

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :  C07K 14/00		A2	(11) International Publication Number: <b>WO 99/47552</b>  (43) International Publication Date: 23 September 1999 (23.09.99)
<p>(21) International Application Number: PCT/EP99/01779</p> <p>(22) International Filing Date: 17 March 1999 (17.03.99)</p> <p>(30) Priority Data: 09/042,763 17 March 1998 (17.03.98) US</p> <p>(71) Applicant (<i>for all designated States except AT US</i>): NOVARTIS AG [CH/CH]; Schwarzwaldallee 215, CH-4058 Basel (CH).</p> <p>(71) Applicant (<i>for AT only</i>): NOVARTIS-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H. [AT/AT]; Brunner Strasse 59, A-1235 Vienna (AT).</p> <p>(72) Inventors; and</p> <p>(75) Inventors/Applicants (<i>for US only</i>): SALMERON, John, Manuel [US/US]; 1308 Blackberry Lane, Hillsborough, NC 27278 (US). WEISLO, Laura, Jean [US/US]; 914 West South Street, Raleigh, NC 27603 (US). STRAWN, Leslie, Jean [US/US]; 32 Damsey Circle, Durham, NC 27713 (US). KRAMER, Catherine, Mae [US/US]; 608 Dana Court, Hillsborough, NC 27278 (US).</p> <p>(74) Agent: BECKER, Konrad; Novartis AG, Corporate Intellectual Property, Patent &amp; Trademark Dept., CH-4002 Basel (CH).</p>			<p>(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).</p> <p><b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i></p>

(54) Title: GENES CONTROLLING DISEASES

## (57) Abstract

This invention describes genes encoding proteins which control resistance of plants to fungal pathogens. The invention also describes transgenic plants resistant to fungal pathogens and methods for making plants resistant to fungal pathogens. The invention further discloses a method to isolate additional genes coding for additional proteins controlling the resistance of plants to fungal pathogens.

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakhstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

## GENES CONTROLLING DISEASES

The invention describes nucleotide sequences encoding proteins controlling resistance of plants to fungal diseases. The invention also relates to plants resistant to fungal diseases, and to methods of making plants resistant to fungal diseases.

Fungal diseases are responsible for yearly losses of about \$ 9.1 bn on agricultural crops in the US, and are caused by a large variety of biologically diverse pathogens. Different strategies have traditionally been used to control them. Resistance traits have been bred into agriculturally important varieties, thus providing various levels of resistance against either a narrow range of pathogen isolates or races, or against a broader range. However, this involves the long and labor intensive process of introducing desirable traits into commercial lines by genetic crosses and, due to the risk of pests evolving to overcome natural plant resistance, a constant effort to breed new resistance traits into commercial lines is required. Alternatively, fungal diseases have been controlled by the application of chemical fungicides. This strategy usually results in efficient control, but is also associated with the possible development of resistant pathogens and can be associated with a negative impact on the environment. Moreover, in certain crops, such as barley and wheat, the control of fungal pathogens by chemical fungicides is difficult or impractical.

Recent techniques have allowed a better understanding of the interactions between plants and their pathogens at the molecular level and mechanisms of resistance have been partially unraveled. While a large portion of this molecular characterization has been conducted in the model plant *Arabidopsis*, resistance mechanisms have also begun to be elucidated in economically important crops.

The powdery mildews are a major disease affecting most plant species and have been widely studied. They are characterized by spots or patches of a white to grayish growth on plant tissues, which correspond to the mycelium and cleistothecia of the fungus. Powdery mildews are caused by several species of fungi of the order Erysiphales. For example, *Erysiphe graminis* causes the powdery mildew of cereals and grasses. While powdery mildews are hard to control in most crops, barley lines resistant to most known isolates of the pathogen are available. It was shown that mutations at a single locus, the *Mlo* locus, are responsible for resistance phenotype. The mechanism of *mlo* resistance has been partially elucidated; it involves the formation of large cell wall appositions, called papillae, at the contact sites with the pathogen, which mainly contain callose, but also carbohydrates,

phenols and proteins. In mlo plants, cell wall appositions prevent the penetration of the pathogen, thus providing resistance.

Unfortunately, this powerful tool to control powdery mildews is restricted to barley. In the view of the problems caused by fungal diseases in agriculture, in particular by powdery mildews, there remains an unfulfilled need for new and effective strategies to control these types of pathogens in other crops, which are economically attractive to the farmers and environmentally acceptable.

The present invention addresses the need for novel disease control strategies in plants via the application of genetic engineering techniques. In particular, this invention relates to control strategies against powdery mildew, preferably in economically important crops.

The present invention relates to isolated DNA molecules encoding Mlo proteins, wherein such Mlo proteins confer resistance of plants to fungal pathogens. In particular, the invention relates to Mlo proteins containing conserved amino acid sequences that the inventors of the present invention are first to discover, and to the isolated DNA molecules encoding such Mlo proteins. The present invention is also drawn to vectors for expression of the DNA molecules of the present invention in plants. The present invention further relates to transgenic plants comprising any one of the DNA molecules of the present invention. The present invention also describes agricultural products with improved phytosanitary properties comprising transgenic plants resistant to fungal pathogens by expression of any of the DNA molecules of the present invention. The present invention also further relates to methods of making plants resistant to fungal diseases by altering the expression in transgenic plants of proteins encoded by the endogenous copies of the genes corresponding to any one of the DNA molecules of the present invention or altering the activity or the stability of proteins encoded by the endogenous copies of the genes corresponding to any one of the DNA molecules of the present invention. Such transgenic plants are desirably resistant to pathogens that infect living epidermal plant cells, more desirably to fungi from the order Erysiphales, also known as powdery mildews, preferably from the genus *Erysiphe*, the causing agent of powdery mildew, more preferably the plants are resistant to *Erysiphe graminis*. The present invention further describes a method to isolate DNA molecules encoding proteins having the same or a similar function as the DNA molecules of the present invention and encoding the conserved amino acid sequences set forth in the present invention.

The present invention thus provides new and effective strategies to control fungal diseases in economically important crops, potentially reducing amounts of chemicals applied to crops and reducing the risk of appearance of pathogens resistant to control agents.

The invention thus provides:

A DNA molecule encoding an Mlo protein conferring upon a plant resistance to fungal pathogens, wherein said protein comprises at least one amino acid sequence identical or substantially similar to an amino acid sequence set forth in SEQ ID No:1 or SEQ ID No:2, wherein said DNA molecule is preferably a cDNA molecule. In a preferred embodiment, the DNA molecule is preferably not derived from barley and is derived from a plant which is either a dicot or from a group of plants consisting of wheat, corn, rice, oats, rye, sorghum, sugarcane, millet, milo, and the palm family. In a preferred embodiment, the DNA molecule of the present invention is identical or substantially similar to any one of the nucleotide sequences set forth in SEQ ID No: 3, SEQ ID No: 5 or SEQ ID No:7, or encodes an Mlo protein identical or substantially similar to an Mlo protein set forth in SEQ ID No: 4, SEQ ID No: 6 or SEQ ID No: 8. In a more preferred embodiment, the DNA molecule comprising the nucleotide sequences set forth in SEQ ID No: 3, SEQ ID No: 5 or SEQ ID No: 7 is derived from wheat. In another preferred embodiment, the DNA molecule of the present invention is identical or substantially similar to any one of the nucleotide sequences set forth in SEQ ID No: 9, SEQ ID No: 11, SEQ ID No: 13, SEQ ID No:15 or SEQ ID No: 17, or encodes an Mlo protein identical or substantially similar to an Mlo protein encoded by any one of the nucleotide sequences set forth in SEQ ID No: 10, SEQ ID No: 12, SEQ ID No: 14, SEQ ID No:16 or SEQ ID No:18. In a more preferred embodiment, the DNA molecule comprising the nucleotide sequences set forth in SEQ ID No: 9, SEQ ID No: 11, SEQ ID No: 13, SEQ ID No:15 or SEQ ID No: 17 is derived from *Arabidopsis thaliana*. In another preferred embodiment, said DNA molecules mentioned hereinbefore are modified such that the activity of the endogenous protein is lost. In one particular embodiment of the present invention, said DNA modification results in one, all or a combination of the following changes in the amino acid sequence of the corresponding protein

- Trp (163) to Arg
- frameshift after Pro (396)
- frameshift after Trp (160)
- Met (1) to Ile

- Gly (227) to Asp
- Met (1) to Val
- Arg (11) to Trp
- missing Phe (183), Thr (184)
- Val (31) to Glu
- Ser (32) to Phe
- Leu (271) to His.

In a further preferred embodiment, the fungal pathogens desirably infect living epidermal cells, more desirably the fungal pathogens are from the order Erysiphales, also known as powdery mildews, preferably from the genus *Erysiphe* and more preferably the fungal pathogen is *Erysiphe graminis*.

In a further embodiment, the isolated DNA molecule is antisense to an isolated molecule as described above, e.g. antisense to a DNA molecule, e.g. a cDNA molecule, encoding an Mlo protein comprising at least one amino acid sequence identical or substantially similar to an amino acid sequences set forth in SEQ ID No:1 or SEQ ID No:2, especially antisense to a DNA molecule identical or substantially similar to a DNA molecule set forth in SEQ ID Nos:3, 5, 7 ,9, 11, 13, 15 or 17 and encoding an Mlo protein identical or substantially similar to an Mlo protein set forth in SEQ ID Nos:4, 6, 8, 10, 12, 14, 16 or 18.

The invention further provides:

A protein comprising at least one amino acid sequence identical or substantially similar to an amino acid sequence set forth in SEQ ID No: 1 or SEQ ID No:2, wherein said protein is an Mlo protein and confers upon a plant resistance to fungal pathogens. The protein is preferably not derived from barley and is derived from a plant which is either a dicot or from a group of plants consisting of wheat, corn, rice, oats, rye, sorghum, sugarcane, millet, milo, and the palm family. In a preferred embodiment, the protein of the present invention is encoded by a nucleotide sequence identical or substantially similar to any one of the nucleotide sequences set forth in SEQ ID No: 3, SEQ ID No: 5 or SEQ ID No:7 or is identical or substantially similar to any one of the Mlo proteins set forth in SEQ ID No: 4, SEQ ID No: 6 or SEQ ID No: 8. In a more preferred embodiment, the protein is derived from wheat. In another preferred embodiment, the protein of the present invention is encoded by a nucleotide sequence identical or substantially similar to any one of the nucleotide sequences set forth in SEQ ID No: 9, SEQ ID No: 11, SEQ ID No: 13, SEQ ID No:15 or

SEQ ID No: 17 or is identical or substantially similar to any one of the Mlo proteins set forth in SEQ ID No: 10, SEQ ID No: 12, SEQ ID No: 14, SEQ ID No:16 or SEQ ID No:18. In a more preferred embodiment, the protein is derived from *A. thaliana*. In another preferred embodiment, the fungal pathogens desirably infect living epidermal cells, more desirably the fungal pathogens are from the order Erysiphales, also known as powdery mildews, preferably from the genus *Erysiphe* and more preferably the fungal pathogen is *Erysiphe graminis*. In a further embodiment, the present invention also encompasses mutated forms or truncated forms of proteins encoded by any of the DNA molecules described above.

**The invention further provides:**

An expression cassette comprising any one of the DNA molecules described above, e.g. a cDNA as described above, wherein the DNA molecule is operably linked to a promoter and to termination signals capable of expressing the DNA molecule in plants. In a preferred embodiment, the expression cassette is heterologous. In a further preferred embodiment, the promoter and the termination signals are eukaryotic. In a further preferred embodiment, the promoter and termination signals are heterologous with respect to the coding region.

**The invention further provides:**

A vector comprising any one of the expression cassettes described above. In a preferred embodiment, the vector is used for transformation of the expression cassette in plants. In another preferred embodiment, the vector of the present invention is used for amplification of any one of the DNA molecules described above.

**The invention further provides:**

A cell comprising an expression cassette or parts of it comprising an isolated DNA molecule of the present invention, wherein said DNA molecule in said expression cassette is expressible in said cell. In a preferred embodiment, the DNA molecule is not derived from barley. In another preferred embodiment, the cell is a plant cell. In a further preferred embodiment, the expression cassette is stably integrated in the genome of the cell or is included in a self-replicating vector and remains in the cell as an extrachromosomal molecule.

**The invention further provides:**

A plant comprising an expression cassette or parts of it comprising an isolated DNA molecule of the present invention. In a preferred embodiment, the DNA molecule is not derived from barley. In another preferred embodiment, the DNA molecule comprised in the expression cassette is expressible in the plant. In another preferred embodiment, the DNA molecule is stably integrated in the plant genome or is included in a self-replicating vector and remains in the cell as an extrachromosomal molecule. In another preferred embodiment, the plant is resistant to fungal pathogens, desirably fungal pathogens which infect living epidermal cells, more desirably the fungal pathogens are from the order Erysiphales, also known as powdery mildews, preferably from the genus *Erysiphe* and more preferably the fungal pathogen is *Erysiphe graminis*.

The inventions also relates to the seed for such a plant, which seed is optionally treated (e.g. primed or coated) and/or packaged, e.g. placed in a bag with instructions for use.

The invention further provides:

Agricultural products comprising a plant comprising an isolated DNA molecule of the present invention. In a preferred embodiment, the agricultural product is used as e.g. feed, food, or silage and does not contain mycotoxins produced by fungal pathogens, such as e.g. aflatoxins. Therefore, the agricultural product has improved phytosanitary properties.

The invention further provides:

A method for making a plant resistant to a fungal pathogen comprising the step of:

- a) expressing in a plant an RNA transcript encoded by any one of the DNA molecules described above in "sense" orientation; or
- b) expressing in a plant an RNA transcript encoded by any one of the DNA molecules described above in "anti-sense" orientation; or
- c) expressing in a plant a ribozyme capable of specifically cleaving a messenger RNA transcript encoded by an endogenous gene corresponding to any one of the DNA molecules described above; or
- d) expressing in a plant an aptamer specifically directed to an endogenous protein encoded by a gene corresponding to any one of the DNA molecules described above; or
- e) expressing in a plant a mutated or a truncated form of any one of the DNA molecules described above, so that it can act as a dominant negative mutant; or
- f) modifying by homologous recombination in a plant at least one chromosomal copy of the gene corresponding to any one of the DNA molecules described above; or

g) modifying by homologous recombination in a plant at least one chromosomal copy of the regulatory elements of a gene corresponding to any one of the DNA molecules described above.

The invention further provides:

A plant obtained by any one of the method described immediately above including the seed for such a plant, which seed is optionally treated (e.g., primed or coated) and/or packaged, e.g. placed in a bag with instructions for use. In another preferred embodiment, the plant obtained is resistant to fungal pathogens, desirably fungal pathogens which infect living epidermal cells, more desirably the fungal pathogens are from the order Erysiphales, also known as powdery mildews, preferably from the genus *Erysiphe* and more preferably the fungal pathogen is *Erysiphe graminis*.

The invention further provides:

An agricultural product with improved phytosanitary properties obtained by any one of the methods described immediately above.

The invention further provides:

A method for isolating DNA molecules encoding Mlo proteins comprising the steps of:

- a) mixing a degenerated oligonucleotide encoding at least six amino acids of SEQ ID No:1 and a degenerated oligonucleotide complementary to a sequence encoding at least six amino acids of SEQ ID No:2, with DNA extracted from a plant under conditions allowing hybridization of said degenerated oligonucleotides to said DNA; and
- b) amplifying a DNA fragment of said plant DNA, wherein said DNA fragment comprises a at its left and right ends nucleotide sequences that can anneal to said degenerated oligonucleotides in step a); and
- c) obtaining a full-length cDNA clone comprising the DNA fragment of step b).

The invention further provides:

A method for producing mutated copies of the nucleotide sequences of the present invention by "in-vitro recombination" or "DNA shuffling". The mutated copies of the nucleotide sequences of the present invention are used to confer improved resistance to fungal pathogens. In a preferred embodiment, the mutant copies of the nucleotide

sequences of the present invention are used to confer resistance to a broader range of pathogens. One such method is described below:

A method for mutagenizing a DNA molecule according to the present invention, wherein said DNA molecule has been cleaved into double-stranded-random fragments of a desired size, and comprising the steps of:

- a) adding to the resultant population of double-stranded random fragments one or more single or double-stranded oligonucleotides, wherein said oligonucleotides comprise an area of identity and an area of heterology to the double-stranded template polynucleotide;
- b) denaturing the resultant mixture of double-stranded random fragments and oligonucleotides into single-stranded fragments;
- c) incubating the resultant population of single-stranded fragments with a polymerase under conditions which result in the annealing of said single-stranded fragments at said areas of identity to form pairs of annealed fragments, said areas of identity being sufficient for one member of a pair to prime replication of the other, thereby forming a mutagenized double-stranded polynucleotide; and
- d) repeating the second and third steps for at least two further cycles, wherein the resultant mixture in the second step of a further cycle includes the mutagenized double-stranded polynucleotide from the third step of the previous cycle, and the further cycle forms a further mutagenized double-stranded polynucleotide.

#### DEFINITIONS

An "isolated DNA molecule" is a nucleotide sequence that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated nucleotide sequence may exist in a purified form or may exist in a non-native environment such as, for example, a transgenic host cell.

A "protein" as defined herein is the entire protein encoded by the corresponding nucleotide sequence, or is a portion of the protein encoded by the corresponding portion of the nucleotide sequence.

An "isolated protein" is a protein that is encoded by an isolated nucleotide sequence and is therefore not a product of nature. An isolated protein may exist in a purified form or may exist in a non-native environment, such as a transgenic host cell, wherein the protein would

not normally expressed or would be expressed in a different form or different amount in an isogenic non-transgenic host cell.

A plant "resistant to a fungal pathogen" has no or lesser symptoms of a fungal infection caused by the fungal , by inhibiting or limiting the ability of the fungal pathogen to grow on the plant. As a consequence, the plant grows better, has higher yields and produces more seeds.

"A protein conferring resistance upon a plant to a fungal pathogen" means that the protein is involved in the regulation of plant genetic pathways responsible for resistance of the plant to the fungal pathogen. The protein may be a positive regulator in that it enhances resistance of the plant to the fungal pathogen, or the protein may be a negative regulator in that it represses resistance of the plant to the fungal pathogen. A particular example of a protein conferring resistance upon a plant to a fungal pathogen is an Mlo protein.

An "Mlo protein" means herein a member of a family of proteins (the Mlo family) having a substantially similar function in a disease resistance pathway and sharing some structural homology. The structural homology can be e.g. that the members of the family share at least one conserved region.

In its broadest sense, the term "substantially similar", when used herein with respect to a nucleotide sequence, means a nucleotide sequence corresponding to a reference nucleotide sequence, wherein the corresponding sequence encodes a polypeptide having substantially the same structure and function as the polypeptide encoded by the reference nucleotide sequence, e.g. where only changes in amino acids not affecting the polypeptide function occur. Desirably the substantially similar nucleotide sequence encodes the polypeptide encoded by the reference nucleotide sequence. The percentage of identity between the substantially similar nucleotide sequence and the reference nucleotide sequence desirably is at least 80%, more desirably 85%, preferably at least 90%, more preferably at least 95%, still more preferably at least 99%.

The term "substantially similar", when used herein with respect to a protein, means a protein corresponding to a reference protein, wherein the protein has substantially the same structure and function as the reference protein, e.g. where only changes in amino acids not

affecting the polypeptide function occur. When used for a protein or an amino acid sequence the percentage of identity between the substantially similar and the reference protein or amino acid sequence desirably is at least 80%, more desirably 85%, preferably at least 90%, more preferably at least 95%, still more preferably at least 99%.

The percentage of sequence identity is determined using computer programs that are based on dynamic programming algorithms. Computer programs that are preferred within the scope of the present invention include the BLAST (Basic Local Alignment Search Tool) search programs designed to explore all of the available sequence databases regardless of whether the query is protein or DNA. Version BLAST 2.0 (Gapped BLAST) of this search tool has been made publicly available on the Internet (currently <http://www.ncbi.nlm.nih.gov/BLAST/>). It uses a heuristic algorithm which seeks local as opposed to global alignments and is therefore able to detect relationships among sequences which share only isolated regions. The scores assigned in a BLAST search have a well-defined statistical interpretation. Said programs are preferably run with optional parameters set to the default values.

The term "gene" refers to a coding sequence and associated regulatory sequences wherein the coding sequence is transcribed into RNA such as mRNA, rRNA, tRNA, snRNA, sense RNA or antisense RNA. Examples of regulatory sequences are promoter sequences, 5' and 3' untranslated sequences and termination sequences. Further elements that may be present are, for example, introns.

"Expression" refers to the transcription and/or translation of an endogenous gene or a transgene in plants. In the case of antisense constructs, for example, expression may refer to the transcription of the antisense DNA only.

"Expression cassette" as used herein means a DNA sequence capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operably linked to the nucleotide sequence of interest which is operably linked to termination signals. It also typically comprises sequences required for proper translation of the nucleotide sequence. The coding region usually codes for a protein of interest but may also code for a functional RNA of interest, for example antisense RNA or a nontranslated RNA that, in the sense or antisense direction, inhibits expression of a particular gene, e.g., antisense RNA. The expression cassette comprising the nucleotide sequence of interest

may be chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. The expression cassette may also be one which is naturally occurring but has been obtained in a recombinant form useful for heterologous expression. Typically, however, the expression cassette is heterologous with respect to the host, i.e., the particular DNA sequence of the expression cassette does not occur naturally in the host cell and must have been introduced into the host cell or an ancestor of the host cell by a transformation event. The expression of the nucleotide sequence in the expression cassette may be under the control of a constitutive promoter or of an inducible promoter which initiates transcription only when the host cell is exposed to some particular external stimulus. In the case of a multicellular organism, such as a plant, the promoter can also be specific to a particular tissue or organ or stage of development.

"Heterologous" as used herein means "of different natural or of synthetic origin" or represent a non-natural state. For example, if a host cell is transformed with a nucleic sequence derived from another organism, particularly from another species, that gene is heterologous with respect to that host cell and also with respect to descendants of the host cell which carry that gene. The transforming nucleic acid may comprise a heterologous promoter, heterologous coding sequence, or heterologous termination sequence. Alternatively, the transforming nucleic acid may be completely heterologous or may comprise any possible combination of heterologous and endogenous nucleic acid sequences. Similarly, heterologous refers to a nucleotide sequence derived from and inserted into the same natural, original cell type, but which is present in a non-natural state, e.g. a different copy number, or under the control of different regulatory elements.

The term "promoter" refers to a DNA sequence that initiates transcription of an associated DNA sequence. The promoter region may also include elements that act as regulators of gene expression such as activators, enhancers, and/or repressors.

"Synthetic nucleotide sequence" as used herein means a nucleotide sequence comprising structural characters that are not present in the natural sequence. For example, an artificial sequence that resembles more closely the G+C content and the normal codon distribution of dicot and/or monocot genes is said to be synthetic.

A regulatory DNA sequence is said to be "operably linked to" or "associated with" a DNA sequence that codes for an RNA or a protein if the two sequences are situated such that the regulatory DNA sequence affects expression of the coding DNA sequence.

"Regulatory elements" refer to sequences involved in conferring the expression of a nucleotide sequence. Regulatory elements comprise a promoter operably linked to the nucleotide sequence of interest and termination signals. They also typically encompass sequences required for proper translation of the nucleotide sequence.

A "plant" refers to any plant or part of a plant and particularly to seed plants at any stage of development. Therein are also included cuttings, cell or tissue cultures and seeds. As used in conjunction with the present invention, the term "plant tissue" includes, but is not limited to, whole plants, plant organs, plant seeds, protoplasts, callus, cell cultures, and any groups of plant cells organized into structural and/or functional units.

A "plant cell" refers to the structural and physiological unit of the plant, comprising a protoplast and a cell wall. The plant cell may be in form of an isolated single cell or a cultured cell, or as a part of higher organized unit such as, for example, a plant tissue, or a plant organ.

"Transformation" as used herein means introduction of a nucleic acid into a cell. In particular, the stable integration of a DNA molecule into the genome of an organism of interest.

A "selectable marker" is conferred by a gene whose expression in a plant cell gives the cell a selective advantage. The selective advantage possessed by the cells transformed with the selectable marker gene may be due to their ability to grow in the presence of a negative selective agent, such as an antibiotic or a herbicide, compared to the growth of non-transformed cells. The selective advantage possessed by the transformed cells, compared to non-transformed cells, may also be due to their enhanced or novel capacity to utilize an added compound as a nutrient, growth factor or energy source. Selectable marker gene also refers to a gene or a combination of genes whose expression in a plant cell gives the cell both, a negative and a positive selective advantage.

A "screenable marker" is conferred by a gene whose expression does not give a selective advantage to a transformed cell, but whose expression makes the transformed cell phenotypically distinct from untransformed cells.

**BRIEF DESCRIPTION OF THE SEQUENCES IN THE SEQUENCE LISTING**

SEQ ID No:1	conserved amino acid sequence 1
SEQ ID No:2	conserved amino acid sequence 2
SEQ ID No:3	nucleotide sequence of wheat Mlo protein TrMlo1
SEQ ID No:4	protein sequence of TrMlo1
SEQ ID No:5	nucleotide sequence of wheat Mlo protein TrMlo2
SEQ ID No:6	protein sequence of TrMlo2
SEQ ID No:7	nucleotide sequence of wheat Mlo protein TrMlo3
SEQ ID No:8	protein sequence of TrMlo3
SEQ ID No:9	nucleotide sequence of Arabidopsis Mlo protein CIB10259
SEQ ID No:10	protein sequence of CIB10259
SEQ ID No:11	nucleotide sequence of Arabidopsis Mlo protein CIB10295
SEQ ID No:12	protein sequence of CIB10295
SEQ ID No:13	nucleotide sequence of Arabidopsis Mlo protein CIB10296
SEQ ID No:14	protein sequence of CIB10296
SEQ ID No:15	nucleotide sequence of Arabidopsis Mlo protein F19850
SEQ ID No:16	protein sequence of F19850
SEQ ID No:17	nucleotide sequence of Arabidopsis Mlo protein U95973
SEQ ID No:18	protein sequence of U95973
SEQ ID No:19	oligonucleotide MLO-1
SEQ ID No:20	oligonucleotide MLO-3
SEQ ID No:21	oligonucleotide MLO-5
SEQ ID No:22	oligonucleotide MLO-7
SEQ ID No:23	oligonucleotide MLO-10
SEQ ID No:24	oligonucleotide MLO-15
SEQ ID No:25	oligonucleotide MLO-26
SEQ ID No:26	oligonucleotide MLO-GSP1

SEQ ID No:27 oligonucleotide MLO-GSP2  
SEQ ID No:28 oligonucleotide ST27  
SEQ ID No:29 oligonucleotide N37544-1  
SEQ ID No:30 oligonucleotide N37544-2  
SEQ ID No:31 oligonucleotide T22146-1  
SEQ ID No:32 oligonucleotide T22146-2  
SEQ ID No:33 oligonucleotide H76041-1  
SEQ ID No:34 oligonucleotide H76041-2  
SEQ ID No:35 oligonucleotide SAS-1  
SEQ ID No:36 oligonucleotide SAS-2  
SEQ ID No:37 oligonucleotide SAS-3  
SEQ ID No:38 oligonucleotide SAS-4  
SEQ ID No:39 oligonucleotide SAS-5  
SEQ ID No:40 oligonucleotide SAS-6  
SEQ ID No:41 oligonucleotide SAS-7  
SEQ ID No:42 oligonucleotide SAS-8

Deposits

Deposited material	Accession number	Date of deposit
pCIB 10259	NRRL B-21945	3/10/98
pCIB 10295	NRRL B-21946	3/10/98
pCIB 10296	NRRL B-21947	3/10/98
TrMlo1	NRRL B-21948	3/10/98
TrMlo1-5	NRRL B-21949	3/10/98
TrMlo2	NRRL B-21950	3/10/98
TrMlo2-5	NRRL B-21951	3/10/98
TrMlo3	NRRL B-21952	3/10/98
TrMlo3-5	NRRL B-21953	3/10/98

All deposits were made with the Northern Regional Research Center, 1815 Northern University Street, Peoria, Illinois 61604, USA.

The present invention relates to DNA molecules encoding Mlo proteins, which confer upon a plant resistance to fungal pathogens. The inventors of the present invention are first to identify conserved amino acid sequences among Mlo proteins. The conserved amino acid sequences of the present invention are conserved between three Mlo proteins derived from wheat and three Mlo proteins derived from *A. thaliana*. These amino acid sequences are also conserved in two predicted *Arabidopsis* Mlo proteins. The first conserved amino acid sequence, which is set forth in SEQ ID No:1, comprises 13 amino acids. The fourth amino acid in SEQ ID No:1 is either L, V or I, its fifth amino acid is either V or L and its seventh amino acid is either F or L. The thirteenth amino acid in SEQ ID No:1 is not I and is preferably either T, S or A. The second conserved amino acid sequence, which is set forth in SEQ ID No:2, comprises 14 amino acids. The first amino acid in SEQ ID No:2 is not M and is preferably either I, V, S or G. Its third amino acid is either F, L or V, its sixth amino acid is either Y or N, its seventh amino acid is either A or V, its eighth amino acid is either L or I, its tenth amino acid is either T or S. The invention encompasses isolated Mlo proteins comprising at least one of the conserved amino acid sequences described above and isolated DNA molecules which encode such Mlo proteins. The invention also encompasses isolated Mlo proteins which comprise both conserved sequences set forth in SEQ ID No: 1 and SEQ ID No:2. In a preferred embodiment, the isolated DNA molecules encoding the Mlo proteins of the present invention are cDNA molecules.

In a further embodiment, the DNA molecules encoding Mlo proteins comprising at least one of the conserved amino acid sequences are not derived from barley. In another embodiment, such DNA molecules are derived from a dicot or from wheat, corn, rice, oats, rye, sorghum, sugarcane, millet, milo, or the palm family. In preferred embodiment, a DNA molecule of the present invention is identical or substantially similar to the DNA molecules set forth in SEQ ID No:3, 5 or 7 and in SEQ ID No:9, 11, 13, 15 or 17 or encode an Mlo protein, which is identical or substantially similar to any one of the Mlo proteins set forth in SEQ ID No:4, 6 or 8 or in SEQ ID No:10, 12, 14, 16, 18. The DNA molecules set forth in SEQ ID No:3, 5 or 7 are derived from wheat and encode the Mlo proteins set forth in SEQ ID No:4, 6 or 8, respectively. The isolation of such DNA molecules is further illustrated in example 1. The DNA molecules set forth in SEQ ID No:9, 11, 13, 15 or 17 are derived from *Arabidopsis* and encode the Mlo proteins set forth in SEQ ID No:10, 12, 14, 16 or 18, respectively. The isolation of such DNA molecules is further illustrated in example 2.

The DNA molecule of SEQ ID No:3 encoding a wheat Mlo protein called TrMlo1 is deposited as strains TrMlo1 and TrMlo1-5 with accession numbers NRRL B-21948 and NRRL B-

21949, respectively. The DNA molecule of SEQ ID No:5 encoding a wheat Mlo protein called TrMlo2 is deposited as strains TrMlo2 and TrMlo2-5 with accession numbers NRRL B-21950 and NRRL B-21951, respectively. The DNA molecule of SEQ ID No:7 encoding a wheat Mlo protein called TrMlo3 is deposited as strains TrMlo3 and TrMlo3-5 with accession numbers NRRL B-21952 and NRRL B-21953, respectively. TrMlo1 and TrMlo3 comprise the full-length cDNAs of the corresponding Mlo genes and also comprise some of the corresponding 5' and 3' untranslated regions. TrMlo2 is the longest cDNA clone of the corresponding gene that was recovered. It comprises the entire coding region with exception of the first methionin (start codon) as deduced from a comparison with TrMlo1 and TrMlo3. TrMlo2 also comprises some of the 3' untranslated region of the corresponding gene.

The DNA molecule of SEQ ID No:9 encoding an Arabidopsis Mlo protein called CIB10259 is deposited as strain pCIB10259 with accession number NRRL B-21945. The DNA molecule of SEQ ID No:11 encoding an Arabidopsis Mlo protein called CIB10295 is deposited as strain pCIB10295 with accession number NRRL B-21946. The DNA molecule of SEQ ID No:13 encoding an Arabidopsis Mlo protein called CIB10296 is deposited as strain pCIB1029 with accession number NRRL B-21947. CIB10259, CIB10295 and CIB10296 comprise the full-length cDNAs of the corresponding Mlo genes and also comprise and also comprise some of the corresponding 5' and 3' untranslated regions. The nucleotide sequences encoding the Arabidopsis Mlo protein family members F19850 and U95973 are obtained from the Genbank. However, for both clones, a predicted amino acid sequence is determined and is found not to match the amino acid sequence predicted in the Genbank entry. The Mlo proteins determined by the inventors of the present invention are therefore novel and not obvious. Both newly predicted proteins contain the conserved amino acid sequences set forth in SEQ ID No:1 and 2 and are therefore encompassed by the present invention as well as the isolated cDNAs encoding them.

An Mlo protein encoded by a DNA molecule of the present invention confer upon a plant resistance to fungal pathogens, desirably fungal pathogens which infect living epidermal plant cells, more desirably fungal pathogens from the order Erysiphales, also known as powdery mildews (Agrios G. (1988) Plant Pathology, Third Edition, Academic Press Inc., in particular p 271). Preferably, an Mlo protein encoded by a DNA molecule of the present invention confer upon a plant resistance from the genus *Erysiphe*, more preferably the fungal pathogen is *Erysiphe graminis*.

The present invention also encompasses recombinant vectors comprising any one of the DNA molecules of this invention. In these vectors, such DNA molecules are preferably comprised in an expression cassette comprising regulatory elements for expression of the DNA molecules in a host cell capable of expressing such DNA molecules. Such regulatory elements are usually a promoter and termination signals and preferably also include elements allowing efficient translation of a protein encoded by a DNA molecule of the present invention. In a preferred embodiment, an expression cassette is heterologous. Such vectors are used for transformation of the expression cassette comprising any one of the DNA molecules of this invention into a host cells. In a preferred embodiment, the expression cassette is stably integrated into the DNA of such host cell. In another preferred embodiment, the expression cassette is comprised in a vector, which is capable of replication in a host cell and remains in the host cell as an extrachromosomal molecule. In a further preferred embodiment, such extrachromosomal replicating molecule is used amplify the DNA molecules of this invention in a host cell. In a preferred embodiment, such host cell is a microorganism, such as a bacteria, in particular *E. coli*. In another preferred embodiment, a host cell is an eukaryotic cells, such as e.g. a yeast cell, an insect cell, or a plant cell.

In a further embodiment, a DNA molecule of the present invention is modified by incorporation of random mutations in a technique known as *in-vitro* recombination or DNA shuffling. This technique is described in Stemmer et al., Nature 370: 389-391 (1994) and US Patent 5,605,793 incorporated herein by reference. Millions of mutant copies of the nucleotide sequences are produced based on the original nucleotide sequence described herein and variants with improved properties, such as increased resistance for fungal pathogens or resistance against a wider range of pathogens are recovered. The method encompasses forming a mutagenized double-stranded polynucleotide from a template double-stranded polynucleotide comprising the nucleotide sequence of this invention, wherein the template double-stranded polynucleotide has been cleaved into double-stranded-random fragments of a desired size, and comprises the steps of adding to the resultant population of double-stranded random fragments one or more single or double-stranded oligonucleotides, wherein said oligonucleotides comprise an area of identity and an area of heterology to the double-stranded template polynucleotide; denaturing the resultant mixture of double-stranded random fragments and oligonucleotides into single-stranded fragments; incubating the resultant population of single-stranded fragments with a polymerase under conditions which result in the annealing of said single-stranded

fragments at said areas of identity to form pairs of annealed fragments, said areas of identity being sufficient for one member of a pair to prime replication of the other, thereby forming a mutagenized double-stranded polynucleotide; and repeating the second and third steps for at least two further cycles, wherein the resultant mixture in the second step of a further cycle includes the mutagenized double-stranded polynucleotide from the third step of the previous cycle, and the further cycle forms a further mutagenized double-stranded polynucleotide. In a preferred embodiment, the concentration of a single species of double-stranded random fragment in the population of double-stranded random fragments is less than 1% by weight of the total DNA. In a further preferred embodiment, the template double-stranded polynucleotide comprises at least about 100 species of polynucleotides. In another embodiment, the size of the double-stranded random fragments is from about 5 bp to 5 kb. In a further embodiment, the fourth step of the method comprises repeating the second and the third steps for at least 10 cycles.

The present invention also encompasses cells comprising a DNA molecule of the present invention, wherein the DNA molecule is not in its natural cellular environment. In a preferred embodiment, such cells are plant cells. In another preferred embodiment, a DNA molecule of the present invention is expressible in such cells and is comprised in an expression cassette which allow their expression in such cells. In a preferred embodiment, the expression cassette is stably integrated into the DNA of such host cell. In another preferred embodiment, the expression cassette is comprised in a vector, which is capable of replication in the cell and remains in the cell as an extrachromosomal molecule.

The present invention also encompasses a plant comprising the plant cells described above. In a further embodiment, the DNA molecules of the present invention are expressible in the plant, and expression of any one of the DNA molecules of the present invention or of a portion thereof in transgenic plants confers resistance upon the transgenic plant against fungal pathogens. In a preferred embodiment, the fungal pathogens desirably infect living epidermal cells, more desirably the fungal pathogens are from the order Erysiphales, also known as powdery mildews, preferably from the genus *Erysiphe* and more preferably the fungal pathogen is *Erysiphe graminis*. The present invention therefore also encompasses transgenic plants made resistant to fungal pathogens by the expression of any one of the DNA molecules of the present invention or of a portion thereof.

Plants transformed in accordance with the present invention may be monocots or dicots and include, but are not limited to, maize, wheat, barley, rye, sweet potato, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, pepper, celery, squash, pumpkin, hemp, zucchini, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tomato, sorghum, sugarcane, sugarbeet, sunflower, rapeseed, clover, tobacco, carrot, cotton, alfalfa, rice, potato, eggplant, cucumber, *Arabidopsis thaliana*, and woody plants such as coniferous and deciduous trees, especially maize, wheat, or sugarbeat.

Once a desired nucleotide sequence has been transformed into a particular plant species, it may be propagated in that species or moved into other varieties of the same species, particularly including commercial varieties, using traditional breeding techniques.

For their expression in transgenic plants, the DNA molecules may require modification and optimization. It is known in the art that all organisms have specific preferences for codon usage, and the codons in the nucleotide sequence comprised in the DNA molecules of the present invention can be changed to conform with specific plant preferences, while maintaining the amino acids encoded thereby. Furthermore, high expression in plants is best achieved from coding sequences which have at least 35% GC content, and preferably more than 45%. Nucleotide sequences which have low GC contents may express poorly due to the existence of ATTAA motifs which may destabilize messages, and AATAAA motifs which may cause inappropriate polyadenylation. Although preferred gene sequences may be adequately expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledons or dicotyledons as these preferences have been shown to differ (*Murray et al. Nucl. Acids Res.* 17: 477-498 (1989)). In addition, the nucleotide sequences are screened for the existence of illegitimate splice sites which cause message truncation. All changes required to be made within the nucleotide sequences such as those described above are made using well known techniques of site directed mutagenesis, PCR, and synthetic gene construction using the methods described in the published patent applications EP 0 385 962, EP 0 359 472 and WO 93/07278.

For efficient initiation of translation, sequences adjacent to the initiating methionine may require modification. For example, they can be modified by the inclusion of sequences known to be effective in plants. Joshi has suggested an appropriate consensus for plants (*NAR* 15: 6643-6653 (1987)) and Clontech suggests a further consensus translation initiator

(1993/1994 catalog, page 210). These consensuses are suitable for use with the nucleotide sequences of this invention. The sequences are incorporated into constructions comprising the nucleotide sequence, up to and including the ATG (whilst leaving the second amino acid unmodified), or alternatively up to and including the GTC subsequent to the ATG (with the possibility of modifying the second amino acid of the transgene).

The DNA molecules in transgenic plants are driven by a promoter shown to be functional in plants. The choice of promoter will vary depending on the temporal and spatial requirements for expression, and also depending on the target species. For the protection of plants against foliar pathogens, expression in leaves is preferred; for the protection of plants against ear pathogens, expression in inflorescences (e.g. spikes, panicles, cobs, etc.) is preferred; for protection of plants against root pathogens, expression in roots is preferred; for protection of seedlings against soil-borne pathogens, expression in roots and/or seedlings is preferred. In many cases, however, protection against more than one type of phytopathogen is sought, and thus expression in multiple tissues is desirable. Although many promoters from dicotyledons have been shown to be operational in monocotyledons and vice versa, ideally dicotyledonous promoters are selected for expression in dicotyledons, and monocotyledonous promoters for expression in monocotyledons. However, there is no restriction to the provenance of selected promoters; it is sufficient that they are operational in driving the expression of the DNA molecules in the desired cell.

Preferred promoters which are expressed constitutively include promoters derived from Agrobacterium opine synthase genes, e.g. the nos promoter, or a dual promoter from the Agrobacterium Ti plasmid (Velten et al. (1984) EMBO J. 3: 2723-2730), or viral promoters operable in plants, e.g. the CaMV 35S and 19S promoters, and promoters from genes encoding actin or ubiquitin. Another preferred promoter is a synthetic promoter, such as the Gelvin Super MAS promoter (Ni et al. (1995) Plant J. 7: 661-676). The DNA molecules of this invention can also be expressed under the regulation of promoters which are chemically regulated. This enables the protein conferring fungal diseases to be synthesized only when the crop plants are treated with the inducing chemicals. Preferred technology for chemical induction of gene expression is detailed in the published application EP 0 332 104 and US patent 5,614,395. A preferred promoter for chemical induction is the tobacco PR-1a promoter.

A preferred category of promoters is that which is wound inducible. Numerous promoters have been described which are expressed at wound sites and also at the sites of

phytopathogen infection. Ideally, such a promoter should only be active locally at the sites of infection, and in this way the protein controlling fungal diseases only accumulates in cells which need to synthesize it to kill the invading insect pest. Preferred promoters of this kind include those described by Stanford *et al.* Mol. Gen. Genet. 215: 200-208 (1989), Xu *et al.* Plant Molec. Biol. 22: 573-588 (1993), Logemann *et al.* Plant Cell 1: 151-158 (1989), Rohrmeier & Lehle, Plant Molec. Biol. 22: 783-792 (1993), Firek *et al.* Plant Molec. Biol. 22: 129-142 (1993), and Warner *et al.* Plant J. 3: 191-201 (1993).

Preferred tissue specific expression patterns include green tissue specific, root specific, stem specific, and flower specific. Promoters suitable for expression in green tissue include many which regulate genes involved in photosynthesis and many of these have been cloned from both monocotyledons and dicotyledons. A preferred promoter is the maize PEPC promoter from the phosphoenol carboxylase gene (Hudspeth & Grula, Plant Molec. Biol. 12: 579-589 (1989)). A preferred promoter for root specific expression is that described by de Framond (FEBS 290: 103-106 (1991); EP 0 452 269) and a further preferred root-specific promoter is that from the T-1 gene provided by this invention. A preferred stem specific promoter is that described in US patent 5,625,136 and which drives expression of the maize *trpA* gene.

Preferred embodiments of the invention are transgenic plants expressing a DNA molecule in a root-specific fashion. Further preferred embodiments are transgenic plants expressing the DNA molecule in a wound-inducible or pathogen infection-inducible manner.

In addition to the selection of a suitable promoter, constructions for expression of the protein in plants require an appropriate transcription terminator to be attached downstream of the heterologous nucleotide sequence. Several such terminators are available and known in the art (e.g. *tm1* from CaMV, E9 from *rbcS*). Any available terminator known to function in plants can be used in the context of this invention.

Numerous other sequences can be incorporated into expression cassettes for the DNA molecules of this invention. These include sequences which have been shown to enhance expression such as intron sequences (e.g. from *Adh1* and *bronze1*) and viral leader sequences (e.g. from TMV, MCMV and AMV).

It may be preferable to target expression of the DNA molecules to different cellular localizations in the plant. In some cases, localization in the cytosol may be desirable, whereas in other cases, localization in some subcellular organelle may be preferred. Subcellular localization of transgene encoded enzymes can be undertaken using

techniques well known in the art. Typically, the DNA encoding the target peptide from a known organelle-targeted gene product is manipulated and fused upstream of the nucleotide sequence. Many such target sequences are known for the chloroplast and their functioning in heterologous constructions has been shown.

Vectors suitable for plant transformation are described elsewhere in this specification. For *Agrobacterium*-mediated transformation, binary vectors or vectors carrying at least one T-DNA border sequence are suitable, whereas for direct gene transfer any vector is suitable and linear DNA containing only the construction of interest may be preferred. In the case of direct gene transfer, transformation with a single DNA species or co-transformation can be used (Schocher *et al.* *Biotechnology* 4: 1093-1096 (1986)). For both direct gene transfer and *Agrobacterium*-mediated transfer, transformation is usually (but not necessarily) undertaken with a selectable marker which may provide resistance to an antibiotic (kanamycin, hygromycin or methotrexate) or a herbicide (glufosinate, glyphosate or a protoporphyrinogen oxidase inhibitor), or a selectable marker which may confer a selective advantage to transformed cells, such as a phospho-mannose isomerase gene. The choice of selectable marker is not, however, critical to the invention.

In another preferred embodiment, the DNA molecules of this invention are directly transformed into the plastid genome. Plastid transformation technology is extensively described in U.S. Patent Nos. 5,451,513, 5,545,817, and 5,545,818, in PCT application no. WO 95/16783, and in McBride *et al.* (1994) *Proc. Natl. Acad. Sci. USA* 91, 7301-7305. The basic technique for chloroplast transformation involves introducing regions of cloned plastid DNA flanking a selectable marker together with the DNA molecule of interest into a suitable target tissue, e.g., using biotics or protoplast transformation (e.g., calcium chloride or PEG mediated transformation). The 1 to 1.5 kb flanking regions, termed targeting sequences, facilitate homologous recombination with the plastid genome and thus allow the replacement or modification of specific regions of the plastome. Initially, point mutations in the chloroplast 16S rRNA and rps12 genes conferring resistance to spectinomycin and/or streptomycin are utilized as selectable markers for transformation (Svab, Z., Hajdukiewicz, P., and Maliga, P. (1990) *Proc. Natl. Acad. Sci. USA* 87, 8526-8530; Staub, J. M., and Maliga, P. (1992) *Plant Cell* 4, 39-45). This resulted in stable homoplasmic transformants at a frequency of approximately one per 100 bombardments of target leaves. The presence of cloning sites between these markers allowed creation of a plastid targeting vector for introduction of foreign genes (Staub, J.M., and Maliga, P. (1993) *EMBO J.* 12, 601-606,

hereby incorporated by reference). Substantial increases in transformation frequency are obtained by replacement of the recessive rRNA or r-protein antibiotic resistance genes with a dominant selectable marker, the bacterial *aadA* gene encoding the spectinomycin-detoxifying enzyme aminoglycoside-3'-adenyltransferase (Svab, Z., and Maliga, P. (1993) *Proc. Natl. Acad. Sci. USA* 90, 913-917). Previously, this marker had been used successfully for high-frequency transformation of the plastid genome of the green alga *Chlamydomonas reinhardtii* (Goldschmidt-Clermont, M. (1991) *Nucl. Acids Res.* 19, 4083-4089). Other selectable markers useful for plastid transformation are known in the art and encompassed within the scope of the invention. Typically, approximately 15-20 cell division cycles following transformation are required to reach a homoplasticidic state. Plastid expression, in which genes are inserted by homologous recombination into all of the several thousand copies of the circular plastid genome present in each plant cell, takes advantage of the enormous copy number advantage over nuclear-expressed genes to permit expression levels that can readily exceed 10% of the total soluble plant protein.

The present invention also encompasses agricultural products, which comprise transgenic plants made resistant to fungal pathogens by expression of any one of the DNA molecules of the present invention or made resistant to fungal pathogens by any one of the methods described *infra*. Since such plants are resistant to fungal pathogens, pathogen growth on them is suppressed. Such plants and the agricultural products derived therefrom are therefore less likely to contain mycotoxins, which are naturally produced by many fungal pathogens and which can be very toxic to human and to animals. Therefore, such agricultural products have better phytosanitary properties. In a preferred embodiment, such agricultural products are used as feedstuff, as silage, or as food.

It is a further object of the present invention, to provide methods of making plants resistant to fungal pathogens. The Mlo proteins encoded by the DNA molecules of the present invention confer upon a plant resistance to a fungal pathogen and it is a preferred object of the present invention to alter the expression of such proteins in their natural host environment. It is a further preferred object of the present invention to alter the stability or the activity of such proteins in their natural environment. Such alterations of the expression, stability, or activity of the proteins encoded by the DNA molecules of the present invention in a plant result in increased resistance of the plant to fungal pathogens. In a preferred embodiment, a protein encoded by a DNA molecule of the present invention the protein is a

negative regulator of resistance of a plant to fungal pathogens, in that it represses genetic pathways in the plant which are responsible for resistance of the plant to fungal pathogens. Therefore, it is a preferred object of the present invention to reduce the expression of the Mlo proteins encoded by the DNA molecules of the present invention in their natural host environment, or to reduce the stability or the activity of such proteins in their natural host environment.

#### "Sense" Suppression

In a preferred embodiment, reduction of the expression of a protein encoded by a DNA molecule of the present invention is obtained by "sense" suppression (referenced in e.g. Jorgensen et al. (1996) Plant Mol. Biol. 31, 957-973). In this case, the entirety or a portion of a DNA molecule of the present invention is comprised in an expression cassette, which is introduced in a host cell, preferably a plant cell, in which the DNA molecule is expressible. The DNA molecule is inserted in the expression cassette in the "sense orientation", meaning that the 5' end of the DNA molecule is adjacent to the promoter in the expression cassette and that the coding strand of the DNA molecule can be transcribed. In a preferred embodiment, the DNA molecule is fully translatable and all the genetic information comprised in the DNA molecule or portion thereof is translated into a protein. In another preferred embodiment, the DNA molecule is partially translatable and a short peptide is translated. In a preferred embodiment, this is achieved by inserting at least one premature stop codon in the DNA molecule, which bring translation to a halt. In another more preferred embodiment, the DNA molecule is transcribed but no translation product is being made. This is usually achieved by removing the start codon, e.g. the "ATG", of the protein encoded by the DNA molecule. In a further preferred embodiment, the expression cassette comprising the DNA molecule or a portion thereof is stably integrated in the genome of the host cell. In another preferred embodiment, the expression cassette comprising the DNA molecule or a portion thereof is comprised in an extrachromosomal replicating molecule. In transgenic plants containing one of the expression cassettes described immediately above, the expression of the gene corresponding to the DNA molecule comprised in the expression cassette is reduced or abolished, leading to reduced levels of the protein or to its absence in the transgenic plants. As a result thereof, the transgenic plants are resistant to fungal pathogens.

#### "Anti-sense" Suppression

In another preferred embodiment, the reduction of the expression of a protein encoded by a DNA molecule of the present invention is obtained by "anti-sense" suppression. The entirety or a portion of a DNA molecule of the present invention is comprised in an expression cassette, wherein the DNA molecule is introduced in a host cell, preferably a plant cell, in which the DNA molecule is expressible. The DNA molecule is inserted in the expression cassette in the "anti-sense orientation", meaning that the 3' end of the DNA molecule is adjacent to the promoter in the expression cassette and that the non-coding strand of the DNA molecule can be transcribed. In a preferred embodiment, the expression cassette comprising the DNA molecule or a portion thereof is stably integrated in the genome of the host cell. In another preferred embodiment, the expression cassette comprising the DNA molecule or a portion thereof is comprised in an extrachromosomal replicating molecule. Several publications describing this approach are cited for further illustration (Green, P. J. et al., Ann. Rev. Biochem. 55:569-597 (1986); van der Krol, A. R. et al., Antisense Nuc. Acids & Proteins, pp. 125-141 (1991); Abel, P. P. et al., Proc. Natl. Acad. Sci. USA 86:6949-6952 (1989); Ecker, J. R. et al., Proc. Natl. Acad. Sci. USA 83:5372-5376 (Aug. 1986)).

#### Homologous Recombination

In another preferred embodiment, at least one genomic copy corresponding to a DNA molecule of the present invention is modified in the genome of the plant by homologous recombination as further illustrated in Paszkowski et al., EMBO Journal 7:4021-26 (1988). This technique uses the property of homologous sequences to recognize each other and to exchange nucleotide sequences between each by a process known in the art as homologous recombination. Homologous recombination can occur between the chromosomal copy of a nucleotide sequence in a cell and an incoming copy of the nucleotide sequence introduced in the cell by transformation. Specific modifications are thus accurately introduced in the chromosomal copy of the nucleotide sequence. In one embodiment, the regulatory elements of the gene encoding a protein of the present invention are modified. The existing regulatory elements are replaced by different regulatory elements, thus reducing expression of the protein, or they are mutated or deleted, thus abolishing the expression of the protein. In another embodiment, the coding region of the protein is modified by deletion of a part of the coding sequence of the entire coding sequence, or by mutation. Expression of a mutated protein can also confer upon the plant increased resistance to fungal pathogens.

In another preferred embodiment, a mutation in the chromosomal copy of a DNA molecule is introduced by transforming a cell with a chimeric oligonucleotide composed of a contiguous stretch of RNA and DNA residues in a duplex conformation with double hairpin caps on the ends. An additional feature of the oligonucleotide is the presence of 2'-O-methylation at the RNA residues. The RNA/DNA sequence is designed to align with the sequence of a chromosomal copy of a DNA molecule of the present invention and to contain the desired nucleotide change. This technique is further illustrated in US patent 5,501,967.

#### Ribozymes

In a further embodiment, the RNA coding for a protein of the present invention is cleaved by a catalytic RNA, or ribozyme, specific for such RNA. The ribozyme is expressed in transgenic plants and results in reduced amounts of RNA coding for the protein of the present invention in plant cells, thus leading to reduced amounts of protein accumulated in the cells and increased resistance of the plant to fungal pathogens. This method is further illustrated in US patent 4,987,071.

#### Dominant-Negative Mutants

In another preferred embodiment, the activity of the proteins encoded by the nucleotide sequences of this invention is changed. This is achieved by expression of dominant negative mutants of the proteins in transgenic plants, leading to the loss of activity of the endogenous protein.

#### Aptamers

In a further embodiment, the activity of a protein encoded by a DNA molecule of the present invention is inhibited by expressing in transgenic plants nucleic acid ligands, so-called aptamers, which specifically bind to the protein. Aptamers are preferentially obtained by the SELEX (Systematic Evolution of Ligands by EXponential Enrichment) method. In the SELEX method, a candidate mixture of single stranded nucleic acids having regions of randomized sequence is contacted with the protein and those nucleic acids having an increased affinity to the target are partitioned from the remainder of the candidate mixture. The partitioned nucleic acids are amplified to yield a ligand enriched mixture. After several iterations a nucleic acid with optimal affinity to the protein is obtained and is used for expression in transgenic plants. This method is further illustrated in US patent 5,270,163.

**Methods for Isolating Nucleotide Sequences Comprising the Conserved Sequences**

The conserved sequences comprised in Mlo proteins encoded by the DNA molecules of the present invention are used for the isolation of other DNA molecules encoding such sequences. In a preferred embodiment, a mixture of degenerated oligonucleotides that contain at least one possible oligonucleotide encoding a sequences set forth in SEQ ID No:1, or in SEQ ID No:2 is produced. The mixture of oligonucleotides encoding the sequences set forth in SEQ ID No:1 and the mixture of oligonucleotides complementary to sequences encoding the sequences set forth in SEQ ID No:2 are used in a PCR amplification reaction with a template DNA of choice. Mixtures of degenerated oligonucleotides are well known in the art and the degree of degeneration is varied as needed. In a preferred embodiment, the template DNA is a sample of total DNA from a plant, wherein such DNA sample is obtained by methods well known in the art. The amplified fragments resulting from the PCR reactions described above are isolated by methods well known in the art and are used to isolate the corresponding full-length cDNAs by screening a cDNA library or by using a RACE protocol, both well known in the art. This method represents a novel and useful strategy to isolate new genes conferring upon plants resistance to fungal pathogens.

The invention will be further described by reference to the following detailed examples. These examples are provided for purposes of illustration only, and are not intended to be limiting unless otherwise specified.

**EXAMPLES****Example 1: Cloning and Sequencing of Mlo Genes from Wheat**

Mlo genes from wheat are cloned using a reverse transcription-PCR approach. RNA is prepared from leaves of wheat cultivar UC703 and used to program reverse transcription using a Stratagene RT-PCR kit. The resulting cDNA is employed in PCR reactions using the following primers:

MLO-26 5' TTC CAG CAC CGG CAC AAG AA 3' (SEQ ID No:25)

- MLO-10 5' AAG AAC TGC CTG AAG AAG GC 3' (SEQ ID No:23)  
MLO-7 5' CAG AAA CTT GTC TCA TCC CTG G 3' (SEQ ID No:22)  
MLO-5 5' ACA GAG ACC ACC TCC TTG GAA 3' (SEQ ID No:21)  
MLO-15 5' CAC CAC CTT CAT GAT GCT CA 3' (SEQ ID No:24)

PCR is performed using the primer pairs listed below, the reactions of which resulted in amplification of fragments of the indicated sizes:

MLO-26 and MLO-10	503 bp
MLO-26 and MLO-7	1481 bp
MLO-5 and MLO-15	650 bp

The fragments are cloned into either pCR2.1 or pCR2.1-TOPO (Invitrogen). Plasmid DNA is prepared from the transformants and subjected to DNA sequencing. Sequencing reveals the existence of three different cDNA sequences with very high similarity to each other. These wheat Mlo genes are called TrMlo1, TrMlo2 and TrMlo3.

An additional wheat Mlo clone is isolated by screening a wheat cDNA library constructed in Lambda-ZAPII vector. To screen this library, mass excision is performed to convert the library into a Bluescript-based collection of cDNA clones. These are grown separately in pools of 80,000 independent clones, and plasmid DNA prepared. PCR reactions are performed on the pooled DNAs using oligonucleotide primers MLO-5 and MLO-15 (see above). Three pools yield a band of the expected size of 650 base pairs. These pools are subsequently fractionated by serial culturing of bacterial clones at lower and lower density, followed at each step by plasmid DNA preparation and PCR on each subpool using primers MLO-5 and MLO-15. After several rounds of fractionation a single clone is isolated which contained an insert with an Mlo sequence. Sequencing the inserts of two of these clones reveals that they contain identical inserts. The sequence of the third clone reveals an insert identical to that of the other two clones, but with 40 additional bases at the 5' end.

Cloning of the remaining portions of the wheat Mlo is accomplished by random amplification of cDNA ends (RACE). RACE reactions are performed on wheat UC703 poly-A+ RNA using a Marathon cDNA amplification kit (Clontech). Poly-A<sup>+</sup> RNA is prepared from total

wheat RNA using an oligo-dT cellulose column (Gibco BRL). Oligonucleotides used for the RACE reactions are:

MLO-GSP1 5' TGG ACC TCT TCA TGT TCG ATC CCA TCT G 3' (SEQ ID No:26)  
MLO-GSP2 5' CCT GAC GCT GTT CCA GAA TGC GTT TCA 3' (SEQ ID No:27)

Amplification using primer MLO-GSP1 and the 5' adapter primer provided in the kit results in a DNA fragment of ~1300 nucleotides. Amplification using primer MLO-GSP2 and the 3' adapter results in a DNA fragment of ~600 nucleotides. The fragments are cloned into pCR2.1-TOPO and called TrMlo1-5, TrMlo2-5 and TrMlo3-5 and comprise the 5' end of the wheat Mlo genes TrMlo1, TrMlo2 and TrMlo3, respectively. Plasmid DNA is prepared from the clones and the plasmid inserts are sequenced.

#### **Example 2: Cloning of Mlo cDNAs from Arabidopsis**

Comparison of the Mlo protein sequence to translations of database entries using the program TBLASTN revealed a number of entries with similarity to Mlo. Of these, full-length cDNAs corresponding to three Arabidopsis EST entries are cloned, with accession numbers H76041, N37544, and T22146. For each EST, oligonucleotides are designed to amplify a sequence corresponding to the EST from an Arabidopsis cDNA library constructed in plasmid pFL61 (Minet et al. (1992) Gene Nov 16; 121(2): 393-396.). The oligonucleotides used are:

N37544-1 5' AAG ATC AAG ATG AGG ACG TGG AAG TCG TGG 3' (SEQ ID No:29)  
N37544-2 5' AGG CTG AAC CAC TGG GGC GCC TCT CAC CAC 3' (SEQ ID No:30)  
T22146-1 5' CAA GTA TAT GAT GCG CGC TCT AGA GGA TGA 3' (SEQ ID No:31)  
T22146-2 5' AGG TTT CAC CAC TAA GTC TCC TTC AAT GGC 3' (SEQ ID No:32)  
H76041-1 5' GAT CAT TCA AGA CTT AGG CTC ACT CAT GAG 3' (SEQ ID No:33)  
H76041-2 5' AAC AGC AAG GAA GAT TAC AAA TGA TGC CCA 3' (SEQ ID No:34)

The primers N37544-1 and N37544-2 amplify a ~500 base pair fragment from DNA prepared from the cDNA library, and the primers T22146-1 and T22146-2 amplify a ~250 base pair fragment. Primers H76041-1 and H76041-2 amplify two fragments of ~350 and a ~300 base pairs. The ~300 base fragment is of the size predicted from the EST sequence, and is used subsequently to diagnose the presence of a cDNA corresponding to the

H76041 EST in the cDNA library. DNA from the library is transformed into *E. coli*, and clones organized into pools of ~20,000 clones each. DNA from individual pools are screened by PCR using the different primer pairs, and positive pools subsequently subfractionated into smaller and smaller clone numbers. Isolation of individual positive clones is accomplished either by carrying this process through to completion, or in some cases by colony hybridization using EST sequences as probes once pool sizes has reached 200 clones or fewer. For ESTs N37544 and T22146, a clone corresponding to the EST is successfully isolated, and the insert sequenced. The cDNA corresponding to EST N37544 is designated CIB10295 in plasmid pCIB10295 and the plasmid containing the cDNA corresponding to EST T22146 (CIB10296) is designated pCIB10296. For these two ESTs, corresponding genomic sequences are available in the form of portions of Arabidopsis BAC clone sequences recently deposited to GenBank. Notably, the protein sequence predicted to be translated from these genomic sequences, as determined by GenBank, does not correspond to the sequence determined by directly sequencing of the cDNAs. Therefore, the amino acid sequence of the genes corresponding to the ESTs N37544 and T22146 are not obvious from the GenBank entry and are only elucidated through cloning and sequencing of the cDNA clones. The clone isolated using primers for the H76041 EST is found to contain not the gene for H76041, but a novel Mlo gene family member as insert. The insert is completely sequenced, and this Mlo gene family member is designated CIB10259 in plasmid pCIB10259.

#### **Example 3: Construction of Vectors for Expression of the Mlo Genes in Wheat**

Two vectors are constructed for "antisense" expression of barley Mlo gene in wheat. PCR is performed using barley cDNA and the primer pair MLO-5 and MLO-7 (see (1) above), and the reaction results in amplification of a 1124 bp fragment which is cloned into pGEM-T (Promega). This fragment is excised from pGEM-T using the enzymes SacII and NotI. The 1124 base pair fragment is cloned into pBluescript-SK(+). The insert is now excised with BamHI and SacI restriction sites and cloned into BamHI-SacI-digested pCIB9806 (described in patent application 08/838,219) in an orientation in which the Mlo coding sequence runs opposite to the maize ubiquitin promoter. This plasmid is designated pCK01.

To construct a vector for "antisense" expression of the entire Mlo gene in wheat, PCR is performed using the primer pair MLO-1 (5' ATG TCG GAC AAA AAA GGG GT 3' (SEQ ID No:19)) and MLO-10 (see (1) above), and the reaction results in amplification of a 635 bp fragment which is cloned into pCR2.1 (Invitrogen). This fragment is excised from pCR2.1

as an EcoRI fragment, and inserted into pGEM-9Zf(-) (Promega). A 320 nucleotide fragment spanning the naturally-occurring SacI site in Mlo to primer site MLO-10 is excised with SacI and BstXI. pCK01 is digested with SacI and BstXI, and the 320 base fragment inserted. To complete construction of the Mlo gene in the monocot expression vector, a 210 nucleotide SacI fragment is excised from the pGEM-9Zf(-) derivative. This fragment contains the 5' end of the Mlo coding sequence, from the primer site MLO-1 to the naturally-occurring SacI site in the Mlo gene. The pCK01 derivative is digested with SacI and the 210 base fragment inserted. Clones are analyzed for orientation of the 210 base fragment in the newly-constructed vector by PCR, using primers MLO-1 and MLO-10. Only clones in which the 210 base fragment is inserted in the antisense orientation relative to the ubiquitin promoter yielded a 530 base pair product corresponding to the 5' end of the Mlo coding sequence. The resulting plasmid contained the entire Mlo coding sequence in "antisense" orientation relative to the ubiquitin promoter, and is designated pCK02.

To construct a vector for expression of the Mlo gene in "sense" orientation, plasmid pCK02 is digested with BamHI to release the Mlo coding sequence as insert. The BamHI fragment is religated back into the pCK02 base vector. Colonies with the Mlo coding sequence in reverse orientation relative to pCK02 are identified by SacI digestion, which yields a 1.8 kb fragment in such clones, as opposed to a 210 base fragment in clones of identical configuration as pCK02. A clone with the Mlo coding sequence in "sense" orientation relative to the maize ubiquitin promoter is selected as designated pCK03.

**Example 4: Construction of Vectors for Expression of the Mlo Genes in Arabidopsis**  
Mlo clones in pCIB10259, pCIB10295 and pCIB10296, along with pCK02 (for the barley Mlo gene) are used in PCR reactions, resulting in bands that carry the full-length gene sequences flanked by BamHI restriction sites. The sequences of the primers used are:

SAS-1: 5' GGA TTA AGA TCT AAT GGC 3' (SEQ ID No:35, for pCIB10295)

SAS-2: 5' CAA AGA TCT TCA TTT CTT AAA AG 3' (SEQ ID No:36, for pCIB10295)

SAS-3: 5' GCG GAT CCA TGT CGG ACA AAA AAG G 3' (SEQ ID No:37, for barley Mlo)

SAS-4: 5' GCG GAT CCT CAT CCC TGG CTG AAG G 3' (SEQ ID No:38, for barley Mlo)

SAS-5: 5' GGA TCC ACC ATG GCC ACA AGA TG 3' (SEQ ID No:39, for pCIB10259)

SAS-6: 5' GGA TCC TTA GTC AAT ATC ATT AGC 3' (SEQ ID No:40, for pCIB10259)

SAS-7: 5' GCG GAT CCA TGG GTC ACG GAG GAG AAG 3' (SEQ ID No:41, for pCIB10269)

SAS-8: 5' GCG GAT CCT CAG TTG TTA TGA TCA GGA 3' (SEQ ID No:42, for pCIB10296)

The bands are cloned into pCR2.1-TOPO, and the inserts sequenced from the resulting plasmids to confirm the absence of mutations introduced by PCR. The plasmids are digested with BamHI, and the inserts purified and cloned into BamHI-digested pPEH28, a shuttle vector containing a copy of the Arabidopsis ubiquitin gene promoter UBQ3 (Norris et al. (1993) Plant Molecular Biology 21: 895-906) immediately downstream of the BamHI site. Clones containing Mlo sequences fused to UBQ3 are identified, and restriction analysis performed to identify clones with the inserts in the "sense" and "antisense" orientations relative to UBQ3. For each Mlo gene, a clone with the insert in "sense" orientation and a clone with the insert in "antisense" orientation is digested with XbaI, and the insert purified and cloned into XbaI-digested pCIB200. This places the UBQ3-Mlo gene fusion between T-DNA borders.

#### **Example 5: Transformation of Wheat and Identification of Expressors**

Wheat is transformed by particle bombardment of immature embryos as described in detail in patent application WO 94/13822. Plantlets are regenerated on media containing Basta and subjected to PCR analysis. For diagnosis of the presence of the Mlo transgenes by PCR, the following primers are used:

MLO-3: 5' ATG CTA CCA CAC GCA GAT CG 3'

ST27: 5' ACT TCT GCA GGT CGA CTC TA 3'

The primer MLO-3 corresponds to a region of the Mlo transgene, while primer ST27 lies within the maize ubiquitin promoter sequence. The use of both Mlo gene and ubiquitin promoter primers in PCR eliminates false positives arising from the use of two Mlo primers, which might prime from the chromosomal copy of the Mlo gene present in wheat.

Plants confirmed to contain Mlo transgenes are subjected to RNA gel blot analysis, to determine whether they contain altered levels of chromosomally-encoded wheat Mlo mRNAs. Poly-A<sup>+</sup> RNAs are prepared from individual transgenic lines, and blotted onto Hybond-N+ filters. Blots are probed with a 530 base fragment corresponding to the 5' end of the Mlo gene. This region is absent from the pCK01 clone; therefore, hybridization to antisense RNA expressed from the transgene in transgenic lines containing pCK01 does not occur. For pCK02 transgenic lines, in which the transgene contains this 5' end

fragment, the probe hybridizes to two bands of differing size. A ~2.5 kb mRNA corresponding to the antisense transgene is distinguished from the 2.0 kb mRNA derived from wheat chromosomally-encoded Mlo genes. The abundance of the 2.0 kb mRNA is monitored as a measurement of the efficiency of gene suppression achieved by the transgene in individual lines.

#### **Example 6: Disease Testing of Transgenic Wheat Lines**

Plants of transgenic and untransformed UC703 (control) wheat lines are grown in the greenhouse until they are two weeks old. Plants are moved to a Percival growth chamber (cycle of 8 hours dark, 16° C, and 16 hours light, 20° C), and inoculated with *Erysiphe graminis f. sp. tritici* by liberal application of spores. The degree of fungal sporulation is scored two weeks after inoculation. Plants are scored 1 (little to no hyphal growth and no visible sporulation), 2 (some hyphal growth and sporulation, but less control plants) or 3 (hyphal growth and sporulation comparable to controls). Transgenic wheat lines expressing Mlo-constructs show increased resistance to the pathogen. An example for results obtained with the antisense barley Mlo construct is shown below.

#### Screening of siblings from transgenic lines R1 and R2 for disease resistance

Siblings of Mlo antisense transformants (T2 seeds) are planted, inoculated with *E. graminis*, and scored for disease resistance.

	R1	R2	UC703 (control)
<u>Seed Planted</u>	24	24	24
<u>Germinated</u>	14	24	21
<u>Disease Score 1</u>	4	2	0
<u>Disease Score 2</u>	0	0	0
<u>Disease Score 3</u>	10	22	21

The fact that a small percentage of the R1 and R2 plants exhibit resistance may be due to the fact that T2 populations were tested which are still segregating for the transgene.

#### **Example 7: Analysis of Arabidopsis Lines Expressing the Mlo Genes**

Derivatives of pCIB200 containing Mlo genes are used to transform *Arabidopsis* ecotype Ws-O by vacuum infiltration (Bechtold, N., Ellis, J. and Pelletier, G. (1993) *C. R. Acad. Sci. Paris* 316, 1194-1199). Progeny are screened by kanamycin selection to identify transformants. For Mlo transgenic lines, plants are identified which expressed Mlo by RNA gel blot analysis. For Mlo genes, transformants are analyzed for alteration in the steady-state level of mRNA accumulation by RNA gel blot analysis. Transformants exhibiting sense or antisense suppression of target genes are assayed for alteration in reaction to the phytopathogenic fungi *Erysiphe cichoracearum* and *Peronospora parasitica*, and the bacterial pathogen *Pseudomonas syringae* pv. *tomato*. Leaves of transgenic plants are inspected both macroscopically and microscopically using trypan blue staining, to test for the presence of necrosis.

For *Erysiphe* inoculation, spores are liberally applied to *Arabidopsis* rosettes and plants are maintained in a Percival growth chamber at 25°C. Degree of fungal sporulation is scored 10 days after inoculation.

**Example 8: Use of Regions of Similarity between Mlo Sequences to Isolate Additional Mlo Gene Family Members**

Alignment of the amino acid sequences predicted to be encoded by Mlo genes revealed a number of short regions of high amino acid similarity between all the gene products. Degenerate primers are designed to these regions, and PCR reactions with these primers are carried out according to PCR reagent supplier's recommendations. The amplified fragments are used as a probe to isolate full-length cDNA or genomic clones of the novel Mlo genes. Amino acid sequences conserved between the Mlo proteins of the present invention (in bold) and degenerated oligonucleotides used for the isolation of additional Mlo genes are shown below:

	E	L	M	X1	X2	G	X3	I	S	L	L	L	X4
<b>WHEAT</b>													
TrMlo1		GAG	CTC	ATG	CTG	GTG	GGC	TTC	ATC				
TrMlo2		GAG	CTG	ATG	CTG	GTG	GGG	TTC	ATC				
TrMlo3		GAG	CTG	ATG	CTG	GTG	GGA	TTC	ATC				

**ARABIDOPSIS**

CIB10259 GAG CTG ATG ATT CTA GGA TTC ATT

CIB10295 GAG CTT ATG CTG TTG GGA TTC ATA  
 CIB10296 GAG CTG ATG TTG TTA GGG TTT ATA  
 F19850 GAG CTG ATG GTT CTT GGA TTC ATC  
 U95973 GAG TTG ATG TTG CTG GGA CTT ATA

5' GAG CTB ATG MTB BTR GGM TTC AT 3'

X5	T	X6	P	L	X7	X8	X9	V	X10	Q	M	G	S
----	---	----	---	---	----	----	----	---	-----	---	---	---	---

**WHEAT**

TrMlo1	GCG CTC GTC ACA CAG ATG GGA TCA
TrMlo2	GCG CTC GTC ACA CAG ATG GGA TCG
TrMlo3	GCG CTA GTC ACA CAG ATG GGA TCA

**ARABIDOPSIS**

CIB10259	GCA CTA GTT ACT CAG ATG GGT TCA
CIB10295	GCA CTT GTT ACT CAG ATG GGT AGT
CIB10296	GCC ATC GTC TCA CAG ATG GGA AGT
F19850	GCA CTC GTA ACT CAG ATG GGT TCT
U95973	GTA ATC GTT ACT CAG ATG GGA TCT

5' WCC CAT CTG AGT GAC DAG BGC RTA 3'

X1= L, V or I, X2= V or L, X3= F or L, X4= T, S or A.

X5= I, V, S or G, X6= F, L or V, X7= Y or N, X8= A or V, X9= L or I, X10= T or S.

R=A,G Y=C,T M=A,C K=G,T S=C,G W=A,T H=A,C,T B=C,G,T V=A,C,G D=A,G,T

N=A,C,G,T.

**Example 9: Modification of Coding Sequences and Adjacent Sequences**

The DNA molecules described in this application can be modified for expression in transgenic plant hosts to achieve and optimize or down-regulate their expression. The following problems may be encountered and the modification of these DNA molecules can be undertaken using techniques well known in the art.

- (1) Codon Usage. The preferred codon usage in some plants differs from the preferred codon usage in some other plant species. Typically plant evolution has tended towards a strong preference of the nucleotides C and G in the third base position of monocotyledons,

whereas dicotyledons often use the nucleotides A or T at this position. By modifying a gene to incorporate preferred codon usage for a particular target transgenic species, many of the problems described below for GC/AT content and illegitimate splicing will be overcome.

(2) GC/AT Content. Plant genes typically have a GC content of more than 35%. DNA molecules which are rich in A and T nucleotides can cause several problems in plants. Firstly, motifs of ATTTA are believed to cause destabilization of messages and are found at the 3' end of many short-lived mRNAs. Secondly, the occurrence of polyadenylation signals such as AATAAA at inappropriate positions within the message is believed to cause premature truncation of transcription. In addition, monocotyledons may recognize AT-rich sequences as splice sites (see below).

(3) Sequences Adjacent to the Initiating Methionine. It is believed that ribosomes attach to the 5' end of the message and scan for the first available ATG at which to start translation. Nevertheless, it is believed that there is a preference for certain nucleotides adjacent to the ATG and that expression of DNA molecules of the present invention can be enhanced by the inclusion of a new consensus translation initiator at the ATG. Clontech (1993/1994 catalog, page 210) have suggested a sequence as a consensus translation initiator for the expression of the *E. coli uidA* gene in plants. Further, Joshi (NAR 15: 6643-6653 (1987)) has compared many plant sequences adjacent to the ATG and suggests a consensus sequence. In situations where difficulties are encountered in the expression of DNA molecules in plants, inclusion of one of these sequences at the initiating ATG may improve translation. In such cases the last three nucleotides of the consensus may not be appropriate for inclusion in the modified sequence due to their modification of the second AA residue. Preferred sequences adjacent to the initiating methionine may differ between different plant species. A survey of 14 maize genes located in the GenBank database provided the following results:

Position Before the Initiating ATG in 14 Maize Genes:

	<u>-10</u>	<u>-9</u>	<u>-8</u>	<u>-7</u>	<u>-6</u>	<u>-5</u>	<u>-4</u>	<u>-3</u>	<u>-2</u>	<u>-1</u>
C	3	8	4	6	2	5	6	0	10	7
T	3	0	3	4	3	2	1	1	1	0
A	2	3	1	4	3	2	3	7	2	3

G     6     3     6     0     6     5     4     6     1     5

This analysis can be done for the desired plant species into which the nucleotide sequence is being incorporated, and the sequence adjacent to the ATG modified to incorporate the preferred nucleotides.

(4) Removal of Illegitimate Splice Sites. DNA molecules of the present invention may also contain motifs which may be recognized in plants as 5' or 3' splice sites, and be cleaved, thus generating truncated or deleted messages. These sites can be removed using the techniques well known in the art.

(5) **Creation of Dominant-Negative Mutants**

In addition, DNA molecules of the present invention may also include molecules that are modified in such a way that the activity of the proteins encoded by the nucleotide sequences of this invention is changed. This is achieved by expression of dominant negative mutants of the proteins in transgenic plants, leading to the loss of activity of the endogenous protein. The location of mutations in the Mlo nucleotide sequence leading to the production of such dominant-negative mutations are listed below. Either a single mutation or a combination of the different mutations listed below can be introduced.

Mutation Number	Description of mutation	Location of mutation in wheat Mlo proteins of SEQ ID Nos: 4, 6 and 8 (aa)	Location of mutation in wheat Mlo genes (nt number)		
			TrMlo1 SEQ ID No:3	TrMlo2 SEQ ID No:5	TrMlo3 SEQ ID No:7
1	T to A	Trp (163) to Arg	662	487	684
2	deletion	frameshift after Pro (396)	1366-1367	1191-1192	1388-1389
3	deletion	frameshift after Trp (160)	656-666	481-491	678-688
4	G to A	Met (1) to Ile	178	3	200
5	G to A	Gly (227) to Asp	855	680	877

6	A to G	Met (1) to Val	176	1	198
7	A to T	Arg (11) to Trp	206	31	228
8	deletion	missing Phe (183), Thr (184)	721-726	546-551	743-748
9	T to A	Val (31) to Glu	267	92	289
10	C to T	Ser (32) to Phe	270	95	292
11	T to A	Leu (271) to His	987	812	1009

Techniques for the modification of coding sequences and adjacent sequences are well known in the art. In cases where the initial expression of a DNA molecule of the present invention is low and it is deemed appropriate to make alterations to the sequence as described above, then the construction of synthetic genes can be accomplished according to methods well known in the art. These are, for example, described in the published patent disclosures EP 0 385 962, EP 0 359 472 and WO 93/07278. In most cases it is preferable to assay the expression of gene constructions using transient assay protocols (which are well known in the art) prior to their transfer to transgenic plants.

#### **Example 10: Construction of Plant Transformation Vectors**

Numerous transformation vectors are available for plant transformation, and the DNA molecules of this invention can be used in conjunction with any such vectors. The selection of vector for use will depend upon the preferred transformation technique and the target species for transformation. For certain target species, different antibiotic or herbicide selection markers may be preferred. Selection markers used routinely in transformation include the *npf1* gene which confers resistance to kanamycin, paromomycin, geneticin and related antibiotics (Vieira and Messing, 1982, Gene 19: 259-268; Bevan et al., 1983, Nature 304:184-187) the bacterial *aadA* gene (Goldschmidt-Clermont, 1991, Nucl. Acids Res. 19: 4083-4089), encoding aminoglycoside 3'-adenylyltransferase and conferring resistance to streptomycin or spectinomycin, the *hph* gene which confers resistance to the antibiotic hygromycin (Blochlinger and Diggelmann, 1984, Mol. Cell. Biol. 4: 2929-2931), and the *dhfr* gene, which confers resistance to methotrexate (Bourouis and Jarry, 1983, EMBO J. 2: 1099-1104). Other markers to be used include a phosphinothricin acetyltransferase gene, which confers resistance to the herbicide phosphinothricin (White et al., 1990, Nucl. Acids Res. 18: 1062; Spencer et al. 1990, Theor. Appl. Genet. 79: 625-631), a mutant EPSP

synthase gene encoding glyphosate resistance (Hinchee et al., 1988, *Bio/Technology* **6**: 915-922), a mutant acetolactate synthase (ALS) gene which confers imidazolione or sulfonylurea resistance (Lee et al., 1988, *EMBO J.* **7**: 1241-1248), a mutant *psbA* gene conferring resistance to atrazine (Smeda et al., 1993, *Plant Physiol.* **103**: 911-917), or a mutant protoporphyrinogen oxidase gene as described in US Patent No. 5,767, 373. Selection markers resulting in positive selection, such as a phosphomannose isomerase gene, as described in US Patent No 5,767,378, are also used. Identification of transformed cells may also be accomplished through expression of screenable marker genes such as genes coding for chloramphenicol acetyl transferase (CAT),  $\beta$ -glucuronidase (GUS), luciferase, and green fluorescent protein (GFP) or any other protein that confers a phenotypically distinct trait to the transformed cell.

#### (1) Construction of Vectors Suitable for *Agrobacterium* Transformation

Many vectors are available for transformation using *Agrobacterium tumefaciens*. These typically carry at least one T-DNA border sequence and include vectors such as pBIN19 (Bevan, *Nucl. Acids Res.* (1984)) and pXYZ. Below the construction of two typical vectors is described.

##### Construction of pCIB200 and pCIB2001

The binary vectors pCIB200 and pCIB2001 are used for the construction of recombinant vectors for use with *Agrobacterium* and is constructed in the following manner. pTJS75kan is created by *NarI* digestion of pTJS75 (Schmidhauser & Helinski, *J Bacteriol.* **164**: 446-455 (1985)) allowing excision of the tetracycline-resistance gene, followed by insertion of an *AccI* fragment from pUC4K carrying an NPTII (Vieira & Messing, *Gene* **19**: 259-268 (1982); Bevan et al., *Nature* **304**: 184-187 (1983); McBride et al., *Plant Molecular Biology* **14**: 266-276 (1990)). *XhoI* linkers are ligated to the *EcoRV* fragment of pCIB7 which contains the left and right T-DNA borders, a plant selectable *nos/nptII* chimeric gene and the pUC polylinker (Rothstein et al., *Gene* **53**: 153-161 (1987)), and the *XhoI*-digested fragment is cloned into *Sall*-digested pTJS75kan to create pCIB200 (see also EP 0 332 104, example 19). pCIB200 contains the following unique polylinker restriction sites: *EcoRI*, *SstI*, *KpnI*, *BglII*, *XbaI*, and *Sall*. pCIB2001 is a derivative of pCIB200 which created by the insertion into the polylinker of additional restriction sites. Unique restriction sites in the polylinker of pCIB2001 are *EcoRI*, *SstI*, *KpnI*, *BglII*, *XbaI*, *Sall*, *MluI*, *BclI*, *AvrII*, *Apal*, *HpaI*, and *StuI*. pCIB2001, in addition to containing these unique restriction sites also has plant and

bacterial kanamycin selection, left and right T-DNA borders for *Agrobacterium*-mediated transformation, the RK2-derived *trfA* function for mobilization between *E. coli* and other hosts, and the *OriT* and *OriV* functions also from RK2. The pCIB2001 polylinker is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

Construction of pCIB10 and Hygromycin Selection Derivatives thereof

The binary vector pCIB10 contains a gene encoding kanamycin resistance for selection in plants, T-DNA right and left border sequences and incorporates sequences from the wide host-range plasmid pRK252 allowing it to replicate in both *E. coli* and *Agrobacterium*. Its construction is described by Rothstein *et al.* (Gene 53: 153-161 (1987)). Various derivatives of pCIB10 have been constructed which incorporate the gene for hygromycin B phosphotransferase described by Gritz *et al.* (Gene 25: 179-188 (1983)). These derivatives enable selection of transgenic plant cells on hygromycin only (pCIB743), or hygromycin and kanamycin (pCIB715, pCIB717).

(2) Construction of Vectors Suitable for non-*Agrobacterium* Transformation.

Transformation without the use of *Agrobacterium tumefaciens* circumvents the requirement for T-DNA sequences in the chosen transformation vector and consequently vectors lacking these sequences can be utilized in addition to vectors such as the ones described above which contain T-DNA sequences. Transformation techniques which do not rely on *Agrobacterium* include transformation via particle bombardment, protoplast uptake (e.g. PEG and electroporation) and microinjection. The choice of vector depends largely on the preferred selection for the species being transformed. Below, the construction of some typical vectors is described.

Construction of pCIB3064

pCIB3064 is a pUC-derived vector suitable for direct gene transfer techniques in combination with selection by the herbicide Basta (or phosphinothricin). The plasmid pCIB246 comprises the CaMV 35S promoter in operational fusion to the *E. coli* GUS gene and the CaMV 35S transcriptional terminator and is described in the PCT published application WO 93/07278. The 35S promoter of this vector contains two ATG sequences 5' of the start site. These sites are mutated using standard PCR techniques in such a way as to remove the ATGs and generate the restriction sites *SspI* and *PvuII*. The new restriction sites are 96 and 37 bp away from the unique *Sall* site and 101 and 42 bp away from the actual start site. The resultant derivative of pCIB246 is designated pCIB3025. The GUS

gene is then excised from pCIB3025 by digestion with *Sall* and *SacI*, the termini rendered blunt and religated to generate plasmid pCIB3060. The plasmid pJIT82 is obtained from the John Innes Centre, Norwich and the a 400 bp *SmaI* fragment containing the *bar* gene from *Streptomyces viridochromogenes* is excised and inserted into the *HpaI* site of pCIB3060 (Thompson *et al.* EMBO J 6: 2519-2523 (1987)). This generated pCIB3064 which comprises the *bar* gene under the control of the CaMV 35S promoter and terminator for herbicide selection, a gene fro ampicillin resistance (for selection in *E. coli*) and a polylinker with the unique sites *SphI*, *PstI*, *HindIII*, and *BamHI*. This vector is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

#### Construction of pSOG19 and pSOG35

pSOG35 is a transformation vector which utilizes the *E. coli* gene dihydrofolate reductase (DHFR) as a selectable marker conferring resistance to methotrexate. PCR is used to amplify the 35S promoter (~800 bp), intron 6 from the maize Adh1 gene (~550 bp) and 18 bp of the GUS untranslated leader sequence from pSOG10. A 250 bp fragment encoding the *E. coli* dihydrofolate reductase type II gene is also amplified by PCR and these two PCR fragments are assembled with a *SacI-PstI* fragment from pBI221 (Clontech) which comprised the pUC19 vector backbone and the nopaline synthase terminator. Assembly of these fragments generated pSOG19 which contains the 35S promoter in fusion with the intron 6 sequence, the GUS leader, the DHFR gene and the nopaline synthase terminator. Replacement of the GUS leader in pSOG19 with the leader sequence from Maize Chlorotic Mottle Virus (MCMV) generated the vector pSOG35. pSOG19 and pSOG35 carry the pUC gene for ampicillin resistance and have *HindIII*, *SphI*, *PstI* and *EcoRI* sites available for the cloning of foreign sequences.

#### **Example 11: Requirements for Construction of Plant Expression Cassettes**

Gene sequences intended for expression in transgenic plants are firstly assembled in expression cassettes behind a suitable promoter and upstream of a suitable transcription terminator.

##### Promoter Selection

The selection of promoter used in expression cassettes will determine the spatial and temporal expression pattern of the transgene in the transgenic plant. Selected promoters will express transgenes in specific cell types (such as leaf epidermal cells, mesophyll cells, root cortex cells) or in specific tissues or organs (roots, leaves or flowers, for example) and

this selection will reflect the desired location of biosynthesis of a DNA molecule of the present invention. Alternatively, the selected promoter may drive expression of the gene under a light-induced or other temporally regulated promoter. A further alternative is that the selected promoter be chemically regulated. This provides the possibility of inducing the expression of the nucleotide sequence only when desired and caused by treatment with a chemical inducer.

#### Transcriptional Terminators

A variety of transcriptional terminators are available for use in expression cassettes. These are responsible for the termination of transcription beyond the transgene and its correct polyadenylation. Appropriate transcriptional terminators and those which are known to function in plants and include the CaMV 35S terminator, the *tm1* terminator, the nopaline synthase terminator, the pea *rbcS* E9 terminator. These can be used in both monocotyledons and dicotyledons.

#### Sequences for the Enhancement or Regulation of Expression

Numerous sequences have been found to enhance gene expression from within the transcriptional unit and these sequences can be used in conjunction with the genes of this invention to increase their expression in transgenic plants.

Various intron sequences have been shown to enhance expression, particularly in monocotyledonous cells. For example, the introns of the maize *Adh1* gene have been found to significantly enhance the expression of the wild-type gene under its cognate promoter when introduced into maize cells. Intron 1 is found to be particularly effective and enhanced expression in fusion constructs with the chloramphenicol acetyltransferase gene (Callis *et al.*, *Genes Develop* **1**: 1183-1200 (1987)). In the same experimental system, the intron from the maize *bronze1* gene had a similar effect in enhancing expression (Callis *et al.*, *supra*). Intron sequences have been routinely incorporated into plant transformation vectors, typically within the non-translated leader.

A number of non-translated leader sequences derived from viruses are also known to enhance expression, and these are particularly effective in dicotyledonous cells. Specifically, leader sequences from Tobacco Mosaic Virus (TMV, the 'Ω-sequence'), Maize Chlorotic Mottle Virus (MCMV), and Alfalfa Mosaic Virus (AMV) have been shown to be effective in enhancing expression (e.g. Gallie *et al.* *Nucl. Acids Res.* **15**: 8693-8711 (1987); Skuzeski *et al.* *Plant Molec. Biol.* **15**: 65-79 (1990))

#### Targeting of the Gene Product Within the Cell

Various mechanisms for targeting gene products are known to exist in plants and the sequences controlling the functioning of these mechanisms have been characterized in some detail. For example, the targeting of gene products to the chloroplast is controlled by a signal sequence found at the aminoterminal end of various proteins and which is cleaved during chloroplast import yielding the mature protein (e.g. Comai *et al.* J. Biol. Chem. 263: 15104-15109 (1988)). These signal sequences can be fused to heterologous gene products to effect the import of heterologous products into the chloroplast (van den Broeck *et al.* Nature 313: 358-363 (1985)). DNA encoding for appropriate signal sequences can be isolated from the 5' end of the cDNAs encoding the RUBISCO protein, the CAB protein, the EPSP synthase enzyme, the GS2 protein and many other proteins which are known to be chloroplast localized.

Other gene products are localized to other organelles such as the mitochondrion and the peroxisome (e.g. Unger *et al.* Plant Molec. Biol. 13: 411-418 (1989)). The cDNAs encoding these products can also be manipulated to effect the targeting of heterologous gene products to these organelles. Examples of such sequences are the nuclear-encoded ATPases and specific aspartate amino transferase isoforms for mitochondria. Targeting to cellular protein bodies has been described by Rogers *et al.* (Proc. Natl. Acad. Sci. USA 82: 6512-6516 (1985)).

In addition sequences have been characterized which cause the targeting of gene products to other cell compartments. Aminoterminal sequences are responsible for targeting to the ER, the apoplast, and extracellular secretion from aleurone cells (Koehler & Ho, Plant Cell 2: 769-783 (1990)). Additionally, aminoterminal sequences in conjunction with carboxyterminal sequences are responsible for vacuolar targeting of gene products (Shinshi *et al.* Plant Molec. Biol. 14: 357-368 (1990)).

By the fusion of the appropriate targeting sequences described above to transgene sequences of interest it is possible to direct the transgene product to any organelle or cell compartment. For chloroplast targeting, for example, the chloroplast signal sequence from the RUBISCO gene, the CAB gene, the EPSP synthase gene, or the GS2 gene is fused in frame to the aminoterminal ATG of the transgene. The signal sequence selected should include the known cleavage site and the fusion constructed should take into account any amino acids after the cleavage site which are required for cleavage. In some cases this requirement may be fulfilled by the addition of a small number of amino acids between the cleavage site and the transgene ATG or alternatively replacement of some amino acids within the transgene sequence. Fusions constructed for chloroplast import can be tested

for efficacy of chloroplast uptake by *in vitro* translation of *in vitro* transcribed constructions followed by *in vitro* chloroplast uptake using techniques described by (Bartlett *et al.* In: Edelmann *et al.* (Eds.) *Methods in Chloroplast Molecular Biology*, Elsevier, pp 1081-1091 (1982); Wasmann *et al.* *Mol. Gen. Genet.* **205**: 446-453 (1986)). These construction techniques are well known in the art and are equally applicable to mitochondria and peroxisomes. The choice of targeting which may be required for the insecticidal toxin. This will usually be cytosolic or chloroplastic, although it may in some cases be mitochondrial or peroxisomal. The expression of the nucleotide sequence may also require targeting to the ER, the apoplast or the vacuole.

The above described mechanisms for cellular targeting can be utilized not only in conjunction with their cognate promoters, but also in conjunction with heterologous promoters so as to effect a specific cell targeting goal under the transcriptional regulation of a promoter which has an expression pattern different to that of the promoter from which the targeting signal derives.

#### **Example 12: Examples of Expression Cassette Construction**

The present invention encompasses the expression of a DNA molecule under the regulation of any promoter which is expressible in plants, regardless of the origin of the promoter. Furthermore, the invention encompasses the use of any plant-expressible promoter in conjunction with any further sequences required or selected for the expression of the DNA molecule. Such sequences include, but are not restricted to, transcriptional terminators, extraneous sequences to enhance expression (such as introns [e.g. *Adh* intron 1], viral sequences [e. g. TMV- $\Omega$ ]), and sequences intended for the targeting of the gene product to specific organelles and cell compartments.

#### Constitutive Expression: the CaMV 35S Promoter

Construction of the plasmid pCGN1761 is described in the published patent application EP 0 392 225. pCGN1761 contains the 'double' 35S promoter and the *tm1* transcriptional terminator with a unique *EcoRI* site between the promoter and the terminator and has a pUC-type backbone. A derivative of pCGN1761 is constructed which has a modified polylinker which includes *NotI* and *XbaI* sites in addition to the existing *EcoRI* site. This derivative is designated pCGN1761ENX. pCGN1761ENX is useful for the cloning of cDNA sequences or gene sequences (including microbial ORF sequences) within its polylinker for the purposes of their expression under the control of the 35S promoter in transgenic plants.

The entire 35S promoter-gene sequence-*tml* terminator cassette of such a construction can be excised by *HindIII*, *SphI*, *Sall*, and *XbaI* sites 5' to the promoter and *XbaI*, *BamHI* and *BglII* sites 3' to the terminator for transfer to transformation vectors such as those described above in example 35. Furthermore, the double 35S promoter fragment can be removed by 5' excision with *HindIII*, *SphI*, *Sall*, *XbaI*, or *PstI*, and 3' excision with any of the polylinker restriction sites (*EcoRI*, *NotI* or *XhoI*) for replacement with another promoter.

Modification of pCGN1761ENX by Optimization of the Translational Initiation Site

For any of the constructions described in this section, modifications around the cloning sites can be made by the introduction of sequences which may enhance translation. This is particularly useful when genes derived from microorganisms are to be introduced into plant expression cassettes as these genes may not contain sequences adjacent to their initiating methionine which may be suitable for the initiation of translation in plants. In cases where genes derived from microorganisms are to be cloned into plant expression cassettes at their ATG it may be useful to modify the site of their insertion to optimize their expression. Modification of pCGN1761ENX is described by way of example to incorporate one of several optimized sequences for plant expression (e.g. Joshi, *supra*).

Expression under a Chemically Regulatable Promoter

This section describes the replacement of the double 35S promoter in pCGN1761ENX with any promoter of choice; by way of example the chemically regulated PR-1a promoter is described. The promoter of choice is preferably excised from its source by restriction enzymes, but can alternatively be PCR-amplified using primers which carry appropriate terminal restriction sites. Should PCR-amplification be undertaken, then the promoter should be resequenced to check for amplification errors after the cloning of the amplified promoter in the target vector. The chemically regulatable tobacco PR-1a promoter is cleaved from plasmid pCIB1004 (see EP 0 332 104, example 21 for construction) and transferred to plasmid pCGN1761ENX. pCIB1004 is cleaved with *NcoI* and the resultant 3' overhang of the linearized fragment is rendered blunt by treatment with T4 DNA polymerase. The fragment is then cleaved with *HindIII* and the resultant PR-1a promoter containing fragment is gel purified and cloned into pCGN1761ENX from which the double 35S promoter has been removed. This is done by cleavage with *XbaI* and blunting with T4 polymerase, followed by cleavage with *HindIII* and isolation of the larger vector-terminator containing fragment into which the pCIB1004 promoter fragment is cloned. This generates a pCGN1761ENX derivative with the PR-1a promoter and the *tml* terminator and an

intervening polylinker with unique *EcoRI* and *NotI* sites. The DNA molecule of the present invention can be inserted into this vector, and the fusion products (*i.e.* promoter-gene-terminator) can subsequently be transferred to any selected transformation vector, including those described in this application.

Constitutive Expression: the Actin Promoter

Several isoforms of actin are known to be expressed in most cell types and consequently the actin promoter is a good choice for a constitutive promoter. In particular, the promoter from the rice *Act1* gene has been cloned and characterized (McElroy *et al.* Plant Cell 2: 163-171 (1990)). A 1.3 kb fragment of the promoter is found to contain all the regulatory elements required for expression in rice protoplasts. Furthermore, numerous expression vectors based on the *Act1* promoter have been constructed specifically for use in monocotyledons (McElroy *et al.* Mol. Gen. Genet. 231: 150-160 (1991)). These incorporate the *Act1*-intron 1, *Adh1* 5' flanking sequence and *Adh1*-intron 1 (from the maize alcohol dehydrogenase gene) and sequence from the CaMV 35S promoter. Vectors showing highest expression are fusions of 35S and the *Act1* intron or the *Act1* 5' flanking sequence and the *Act1* intron. Optimization of sequences around the initiating ATG (of the GUS reporter gene) also enhanced expression. The promoter expression cassettes described by McElroy *et al.* (Mol. Gen. Genet. 231: 150-160 (1991)) can be easily modified for the expression of the DNA molecules of the present invention and are particularly suitable for use in monocotyledonous hosts. For example, promoter containing fragments can be removed from the McElroy constructions and used to replace the double 35S promoter in pCGN1761ENX, which is then available for the insertion of specific gene sequences. The fusion genes thus constructed can then be transferred to appropriate transformation vectors. In a separate report the rice *Act1* promoter with its first intron has also been found to direct high expression in cultured barley cells (Chibbar *et al.* Plant Cell Rep. 12: 506-509 (1993)).

Constitutive Expression: the Ubiquitin Promoter

Ubiquitin is another gene product known to accumulate in many cell types and its promoter has been cloned from several species for use in transgenic plants (*e.g.* sunflower - Binet *et al.* Plant Science 79: 87-94 (1991), maize - Christensen *et al.* Plant Molec. Biol. 12: 619-632 (1989)). The maize ubiquitin promoter has been developed in transgenic monocot systems and its sequence and vectors constructed for monocot transformation are disclosed in the patent publication EP 0 342 926. Further, Taylor *et al.* (Plant Cell Rep. 12: 491-495 (1993))

describe a vector (pAHC25) which comprises the maize ubiquitin promoter and first intron and its high activity in cell suspensions of numerous monocotyledons when introduced via microprojectile bombardment. The ubiquitin promoter is clearly suitable for the expression of a DNA molecule of the present invention in transgenic plants, especially monocotyledons. Suitable vectors are derivatives of pAHC25 or any of the transformation vectors described in this application, modified by the introduction of the appropriate ubiquitin promoter and/or intron sequences.

#### Root Specific Expression

A preferred pattern of expression for the nucleotide sequence of the instant invention is root expression. Root expression is particularly useful for the control of soil-borne fungal pathogens. A suitable root promoter is that described by de Framond (FEBS 290: 103-106 (1991)) and also in the published patent application EP 0 452 269. This promoter is transferred to a suitable vector such as pCGN1761ENX for the insertion of the nucleotide sequence and subsequent transfer of the entire promoter-gene-terminator cassette to a transformation vector of interest.

#### Wound Inducible Promoters

Wound-inducible promoters are particularly suitable for the expression of a DNA molecules of the present invention because they are typically active not just on wound induction, but also at the sites of phytopathogen infection. Numerous such promoters have been described (e.g. Xu *et al.* Plant Molec. Biol. 22: 573-588 (1993), Logemann *et al.* Plant Cell 1: 151-158 (1989), Rohrmeier & Lehle, Plant Molec. Biol. 22: 783-792 (1993), Firek *et al.* Plant Molec. Biol. 22: 129-142 (1993), Warner *et al.* Plant J. 3: 191-201 (1993)) and all are suitable for use with the instant invention. Logemann *et al.* (*supra*) describe the 5' upstream sequences of the dicotyledonous potato *wun1* gene. Xu *et al.* (*supra*) show that a wound inducible promoter from the dicotyledon potato (*pin2*) is active in the monocotyledon rice. Further, Rohrmeier & Lehle (*supra*) describe the cloning of the maize *Wip1* cDNA which is wound induced and which can be used to isolated the cognate promoter using standard techniques. Similarly, Firek *et al.* (*supra*) and Warner *et al.* (*supra*) have described a wound induced gene from the monocotyledon *Asparagus officinalis* which is expressed at local wound and pathogen invasion sites. Using cloning techniques well known in the art, these promoters can be transferred to suitable vectors, fused to the DNA molecule of this invention, and used to express these genes at the sites of phytopathogen infection.

#### Pith Preferred Expression

Patent Application WO 93/07278 (to Ciba-Geigy) describes the isolation of the maize *trpA* gene which is preferentially expressed in pith cells. The gene sequence and promoter extend up to -1726 from the start of transcription are presented. Using standard molecular biological techniques, this promoter or parts thereof, can be transferred to a vector such as pCGN1761 where it can replace the 35S promoter and be used to drive the expression of a DNA molecule of this invention in a pith-preferred manner. In fact fragments containing the pith-preferred promoter or parts thereof can be transferred to any vector and modified for utility in transgenic plants.

#### Pollen-Specific Expression

Patent Application WO 93/07278 (to Ciba-Geigy) further describes the isolation of the maize calcium-dependent protein kinase (CDPK) gene which is expressed in pollen cells. The gene sequence and promoter extend up to 1400 bp from the start of transcription. Using standard molecular biological techniques, this promoter or parts thereof, can be transferred to a vector such as pCGN1761 where it can replace the 35S promoter and be used to drive the expression of a DNA molecule of this invention. In fact fragments containing the pollen-specific promoter or parts thereof can be transferred to any vector and modified for utility in transgenic plants.

#### Leaf-Specific Expression

A maize gene encoding phosphoenol carboxylase (PEPC) has been described by Hudspeth & Grula (Plant Molec Biol 12: 579-589 (1989)). Using standard molecular biological techniques the promoter for this gene can be used to drive the expression of any gene in a leaf-specific manner in transgenic plants.

#### Expression with Chloroplast Targeting

Chen & Jagendorf (J. Biol. Chem. 268: 2363-2367 (1993)) have described the successful use of a chloroplast transit peptide for import of a heterologous transgene. This peptide used is the transit peptide from the *rbcS* gene from *Nicotiana plumbaginifolia* (Poulsen *et al.* Mol. Gen. Genet. 205: 193-200 (1986)). Using the restriction enzymes *Dra*I and *Sph*I, or *Tsp*509I and *Sph*I the DNA sequence encoding this transit peptide can be excised from plasmid prbcS-8B (Poulsen *et al. supra*) and manipulated for use with any of the constructions described above. The *Dra*I-*Sph*I fragment extends from -58 relative to the initiating *rbcS* ATG to, and including, the first amino acid (also a methionine) of the mature peptide immediately after the import cleavage site, whereas the *Tsp*509I-*Sph*I fragment

extends from -8 relative to the initiating *rbcS* ATG to, and including, the first amino acid of the mature peptide. Thus, these fragment can be appropriately inserted into the polylinker of any chosen expression cassette generating a transcriptional fusion to the untranslated leader of the chosen promoter (e.g. 35S, PR-1a, actin, ubiquitin etc.), whilst enabling the insertion of the DNA molecule of this invention in correct fusion downstream of the transit peptide. Constructions of this kind are routine in the art. For example, whereas the *Dra*I end is already blunt, the 5' *Tsp509I* site may be rendered blunt by T4 polymerase treatment, or may alternatively be ligated to a linker or adaptor sequence to facilitate its fusion to the chosen promoter. The 3' *Sph*I site may be maintained as such, or may alternatively be ligated to adaptor or linker sequences to facilitate its insertion into the chosen vector in such a way as to make available appropriate restriction sites for the subsequent insertion of the DNA molecule of this invention. Ideally the ATG of the *Sph*I site is maintained and comprises the first ATG of the DNA molecule of this invention. Chen & Jagendorf (*supra*) provide consensus sequences for ideal cleavage for chloroplast import, and in each case a methionine is preferred at the first position of the mature protein. At subsequent positions there is more variation and the amino acid may not be so critical. In any case, fusion constructions can be assessed for efficiency of import *in vitro* using the methods described by Bartlett *et al.* (In: Edelmann *et al.* (Eds.) *Methods in Chloroplast Molecular Biology*, Elsevier. pp 1081-1091 (1982)) and Wasmann *et al.* (*Mol. Gen. Genet.* **205**: 446-453 (1986)). Typically the best approach may be to generate fusions using the DNA molecule of this invention with no modifications at the aminoterminal, and only to incorporate modifications when it is apparent that such fusions are not chloroplast imported at high efficiency, in which case modifications may be made in accordance with the established literature (Chen & Jagendorf, *supra*; Wasman *et al.*, *supra*; Ko & Ko, *J. Biol. Chem.* **267**: 13910-13916 (1992)).

Similar manipulations can be undertaken to utilize other GS2 chloroplast transit peptide encoding sequences from other sources (monocotyledonous and dicotyledonous) and from other genes. In addition, similar procedures can be followed to achieve targeting to other subcellular compartments such as mitochondria.

***What is claimed is:***

1. A DNA molecule encoding an Mlo protein, wherein said Mlo protein comprises at least one of the sequences set forth in SEQ ID No:1 or SEQ ID No:2 and wherein said Mlo protein confers upon a plant resistance to a fungal pathogen.
2. The DNA molecule of claim 1, wherein said DNA molecule is identical or substantially similar to any one of the nucleotide sequences set forth in SEQ ID No:3, SEQ ID No:5 or SEQ ID No:7, or encodes an Mlo protein identical or substantially similar to an Mlo protein set forth in SEQ ID No:4, SEQ ID No:6 or SEQ ID No:8.
3. The DNA molecule of claim 1, wherein said DNA molecule is identical or substantially similar to any one of the nucleotide sequences set forth in SEQ ID No:9, SEQ ID No:11, SEQ ID No:13, SEQ ID No:15 or SEQ ID No:17, or encodes an Mlo protein identical or substantially similar to an Mlo protein encoded set forth in SEQ ID No:10, SEQ ID No:12, SEQ ID No:14, SEQ ID No:16 or SEQ ID No:18.
4. The DNA molecule of any one of claims 1 to 3, wherein said DNA molecule is not derived from barley.
5. The DNA molecule of any one of claims 1 to 4, wherein said DNA is modified such that the activity of the endogenous protein is lost.
6. The DNA molecule of claim 5, wherein the DNA modification results in one, all or a combination of the following changes in the amino acid sequence of the corresponding protein
  - Trp (163) to Arg
  - frameshift after Pro (396)
  - frameshift after Trp (160)
  - Met (1) to Ile
  - Gly (227) to Asp
  - Met (1) to Val
  - Arg (11) to Trp

— missing Phe (183), Thr (184)

— Val (31) to Glu

— Ser (32) to Phe

— Leu (271) to His

7. A DNA molecule antisense to a DNA molecule of any one of claims 1 to 6.

8. A protein comprising at least one of the sequences set forth in SEQ ID No:1 or SEQ ID No:2, wherein said protein is an Mlo protein and confers upon a plant resistance to a fungal pathogen.

9. The protein of claim 8, wherein said protein is encoded by a nucleotide sequence identical or substantially similar to any one of the sequences set forth in SEQ ID No:3, SEQ ID No:5 or SEQ ID No:7, or is identical or substantially similar to any one of the proteins set forth in SEQ ID No:4, SEQ ID No:6 or SEQ ID No:8.

10. The protein of claim 8, wherein said protein is encoded by a nucleotide sequence identical or substantially similar to any one of the sequences set forth in SEQ ID No:9, SEQ ID No:11, SEQ ID No:13, SEQ ID No:15 or SEQ ID No:17, or is identical or substantially similar to any one of the proteins set forth in SEQ ID No:10, SEQ ID No:12, SEQ ID No:14, SEQ ID No:16 or SEQ ID No:18.

11. The protein of any one of claims 8 to 10, wherein said protein is not derived from barley.

12. An expression cassette comprising a DNA molecule of any one of claims 1 to 7.

13. A vector comprising an expression cassette comprising a DNA molecule of claim 12.

14. A cell comprising an expression cassette or parts of it comprising a DNA molecule of any one of claims 1 to 7, wherein said DNA molecule in said expression cassette is expressible in said cell.

15. The cell of claim 14, wherein said DNA molecule is stably integrated in the genome of said cell.
16. The cell of any one of claims 14 or 15, wherein said cell is a plant cell.
17. A plant comprising an expression cassette or parts of it comprising a DNA molecule of any one of claims 1 to 7, wherein said DNA molecule in said expression cassette is expressible in said plant.
18. The plant of claim 17, wherein said DNA molecule is stably integrated in the plant genome of said plant.
19. An agricultural product comprising a plant comprising an isolated DNA molecule of any one of claims 1 to 7, wherein said agricultural product has improved phytosanitary properties.
20. A method for making a plant resistant to a fungal pathogen comprising the steps of:
  - a) expressing in said plant a DNA molecule of any one of claims 1 to 6 in "sense" orientation; or
  - b) expressing in said plant a DNA molecule of any one of claims 1 to 6 in "anti-sense" orientation; or
  - c) expressing in said plant a ribozyme capable of specifically cleaving a messenger RNA transcript encoded by an endogenous gene corresponding to a DNA molecule of any one of claims 1 to 6; or
  - d) expressing in a plant an aptamer specifically directed to a protein or part of a protein encoded by a DNA molecule of any one of claims 1 to 6; or
  - e) expressing in a plant a mutated or a truncated form of a DNA molecule of any one of claims 1 to 6; or
  - f) modifying by homologous recombination in a plant at least one chromosomal copy of the gene corresponding to a DNA molecule of any one of claims 1 to 6.
21. A plant made resistant to a fungal pathogen by the method of claim 20.

22. The plant of claim 21, wherein said fungal pathogen infects living epidermal cells.
23. The plant of claim 21, wherein said fungal pathogen is from the order Erysiphales.
24. The plant of claim 21, wherein said fungal pathogen is from the genus *Erysiphe*.
25. The plant of claim 21, wherein said fungal pathogen is *Erysiphe graminis*.
26. An agricultural product with improved phytosanitary properties obtained using the method of claim 20.
27. A method for isolating DNA molecules encoding Mlo proteins comprising the steps of:
  - a) mixing a degenerated oligonucleotide encoding at least six amino acids of SEQ ID No:1 and a degenerated oligonucleotide complementary to a sequence encoding at least six amino acids of SEQ ID No:2, with DNA extracted from a plant under conditions allowing hybridization of said degenerated oligonucleotides to said DNA; and
  - b) amplifying a DNA fragment of said plant DNA, wherein said DNA fragment comprises at its left and right ends nucleotide sequences that can anneal to said degenerated oligonucleotides in step a); and
  - c) obtaining a full-length cDNA clone comprising the DNA fragment of step b).
28. A method for mutagenizing a DNA molecule of claim 1, wherein said DNA molecule has been cleaved into double-stranded-random fragments of a desired size, and comprising the steps of:
  - a) adding to the resultant population of double-stranded random fragments one or more single or double-stranded oligonucleotides, wherein said oligonucleotides comprise an area of identity and an area of heterology to the double-stranded template polynucleotide;
  - b) denaturing the resultant mixture of double-stranded random fragments and oligonucleotides into single-stranded fragments;
  - c) incubating the resultant population of single-stranded fragments with a polymerase under conditions which result in the annealing of said single-stranded fragments at said areas of identity to form pairs of annealed fragments, said areas of identity being

sufficient for one member of a pair to prime replication of the other, thereby forming a mutagenized double-stranded polynucleotide; and

- d) repeating the second and third steps for at least two further cycles, wherein the resultant mixture in the second step of a further cycle includes the mutagenized double-stranded polynucleotide from the third step of the previous cycle, and the further cycle forms a further mutagenized double-stranded polynucleotide.

- 1 -

## SEQUENCE LISTING

&lt;110&gt; Novartis

&lt;120&gt; Genes controlling diseases

&lt;130&gt; S-30431/A

&lt;140&gt; CGC1989

&lt;141&gt; 1998-03-17

&lt;150&gt; US 09/042763

&lt;151&gt; 1998-03-17

&lt;160&gt; 42

&lt;170&gt; PatentIn Ver. 2.0

&lt;210&gt; 1

&lt;211&gt; 13

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Conserved  
amino acid sequence

&lt;400&gt; 1

Glu Leu Met Xaa Xaa Gly Xaa Ile Ser Leu Leu Leu Xaa  
1 5 10

&lt;210&gt; 2

&lt;211&gt; 14

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Conserved  
amino acid sequence

&lt;400&gt; 2

Xaa Thr Xaa Pro Leu Xaa Xaa Xaa Val Xaa Gln Met Gly Ser  
1 5 10

&lt;210&gt; 3

&lt;211&gt; 1868

&lt;212&gt; DNA

&lt;213&gt; Triticum sp.

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (176) .. (1777)

- 2 -

<220>  
<221> misc\_feature  
<222> (365)..(403)  
<223> location of the amino acid sequence set forth in  
SEQ ID No: 1

<220>  
<221> misc\_feature  
<222> (1352)..(1393)  
<223> location of the amino acid sequence set forth in  
SEQ ID No: 2

<400> 3  
cacgcccaca cttcgccaaac acacaacgta cctgcgtacg tacgctttcc atttccttcc 60  
ttgctccggc cgcccccca cgtagaatag ataccggcc aggttaggtac ctgcgttggat 120  
cagacgaccg gccccgtgggt ctccggacaa ggaaagaggt tgcgctcggg gaccg atg 178  
Met  
1

gcg gac gac gac gag tac ccc cca gcg agg acg ctg ccg gag acg ccg 226  
Ala Asp Asp Asp Glu Tyr Pro Pro Ala Arg Thr Leu Pro Glu Thr Pro  
5 10 15

tcc tgg gcg gtg gcc ctc gtc ttc gcc gtc atg atc atc gtg tcc gtc 274  
Ser Trp Ala Val Ala Leu Val Phe Ala Val Met Ile Ile Val Ser Val  
20 25 30

ctc ctg gag cac gcg ctc cat aag ctc ggc cat tgg ttc cac aag cgg 322  
Leu Leu Glu His Ala Leu His Lys Leu Gly His Trp Phe His Lys Arg  
35 40 45

cac aag aac gcg ctg gcg gag gcg ctg gag aag atc aag gcg gag ctc 370  
His Lys Asn Ala Leu Ala Glu Ala Leu Glu Lys Ile Lys Ala Glu Leu  
50 55 60 65

atg ctg gtg ggc ttc atc tcg ctg ctc gcc gtg acg cag gac ccc 418  
Met Leu Val Gly Phe Ile Ser Leu Leu Ala Val Thr Gln Asp Pro  
70 75 80

atc tcc ggg ata tgc atc tcc gag aag gcc gcc agc atc atg cgg ccc 466  
Ile Ser Gly Ile Cys Ile Ser Glu Lys Ala Ala Ser Ile Met Arg Pro  
85 90 95

tgc aag ctg ccc cct ggc tcc gtc aag agc aag tac aaa gac tac tac 514  
Cys Lys Leu Pro Pro Gly Ser Val Lys Ser Lys Tyr Lys Asp Tyr Tyr  
100 105 110

tgc gcc aaa cag ggc aag gtg tcg ctc atg tcc acg ggc agc ttg cac 562  
Cys Ala Lys Gln Gly Lys Val Ser Leu Met Ser Thr Gly Ser Leu His  
115 120 125

cag ctg cac ata ttc atc ttc gtg ctc gcc gtc ttc cat gtc acc tac 610  
Gln Leu His Ile Phe Ile Phe Val Leu Ala Val Phe His Val Thr Tyr

- 3 -

130	135	140	145	
agc gtc atc atc atg gct cta agc cgt ctc aaa atg aga acc tgg aag Ser Val Ile Ile Met Ala Leu Ser Arg Leu Lys Met Arg Thr Trp Lys				658
150		155		160
aaa tgg gag aca gag acc gcc tcc ctg gaa tac cag ttc gca aat gat Lys Trp Glu Thr Glu Thr Ala Ser Leu Glu Tyr Gln Phe Ala Asn Asp				706
165		170		175
cct ggc cgg ttc cgc ttc acg cac cag acg tcg ttc gtg aag cgg cac Pro Ala Arg Phe Arg Phe Thr His Gln Thr Ser Phe Val Lys Arg His				754
180		185		190
ctg ggc ctc tcc agc acc ccc ggc gtc aga tgg gtg gtg gcc ttc ttc Leu Gly Leu Ser Ser Thr Pro Gly Val Arg Trp Val Val Ala Phe Phe				802
195		200		205
agg cag ttc ttc agg tcg gtc acc aag gtg gac tac ctc acc ttg agg Arg Gln Phe Phe Arg Ser Val Thr Lys Val Asp Tyr Leu Thr Leu Arg				850
210		215		220
gca ggc ttc atc aac gcg cat ttg tcg cat aac agc aag ttc gac ttc Ala Gly Phe Ile Asn Ala His Leu Ser His Asn Ser Lys Phe Asp Phe				898
230		235		240
cac aag tac atc aag agg tcc atg gag gac gac ttc aaa gtc gtc gtt His Lys Tyr Ile Lys Arg Ser Met Glu Asp Asp Phe Lys Val Val Val				946
245		250		255
ggc atc agc ctc ccg ctg tgg tgt gtg gcg atc ctc acc ctc ttc ctt Gly Ile Ser Leu Pro Leu Trp Cys Val Ala Ile Leu Thr Leu Phe Leu				994
260		265		270
gac att gac ggg atc ggc acg ctc acc tgg att tct ttc atc cct ctc Asp Ile Asp Gly Ile Gly Thr Leu Thr Trp Ile Ser Phe Ile Pro Leu				1042
275		280		285
gtc atc ctc ttg tgt gtt gga acc aag ctg gag atg atc atc atg gag Val Ile Leu Leu Cys Val Gly Thr Lys Leu Glu Met Ile Ile Met Glu				1090
290		295		300
atg gcc ctg gag atc cag gac cgg gcg agc gtc atc aag ggg gcg ccc Met Ala Leu Glu Ile Gln Asp Arg Ala Ser Val Ile Lys Gly Ala Pro				1138
310		315		320
gtg gtt gag ccc agc aac aag ttc ttc tgg ttc cac cgc ccc gac tgg Val Val Glu Pro Ser Asn Lys Phe Phe Trp Phe His Arg Pro Asp Trp				1186
325		330		335
gtc ctc ttc ata cac ctg acg cta ttc cag aac gcg ttt cag atg Val Leu Phe Phe Ile His Leu Thr Leu Phe Gln Asn Ala Phe Gln Met				1234
340		345		350
gca cat ttc gtg tgg aca gtg gcc acg ccc ggc ttg aag aaa tgc ttc				1282

- 4 -

<210> 4  
<211> 534  
<212> PRT  
<213> Triticum sp.

- 5 -

&lt;400&gt; 4

Met Ala Asp Asp Asp Glu Tyr Pro Pro Ala Arg Thr Leu Pro Glu Thr  
1 5 10 15

Pro Ser Trp Ala Val Ala Leu Val Phe Ala Val Met Ile Ile Val Ser  
20 25 30

Val Leu Leu Glu His Ala Leu His Lys Leu Gly His Trp Phe His Lys  
35 40 45

Arg His Lys Asn Ala Leu Ala Glu Ala Leu Glu Lys Ile Lys Ala Glu  
50 55 60

Leu Met Leu Val Gly Phe Ile Ser Leu Leu Ala Val Thr Gln Asp  
65 70 75 80

Pro Ile Ser Gly Ile Cys Ile Ser Glu Lys Ala Ala Ser Ile Met Arg  
85 90 95

Pro Cys Lys Leu Pro Pro Gly Ser Val Lys Ser Lys Tyr Lys Asp Tyr  
100 105 110

Tyr Cys Ala Lys Gln Gly Lys Val Ser Leu Met Ser Thr Gly Ser Leu  
115 120 125

His Gln Leu His Ile Phe Ile Phe Val Leu Ala Val Phe His Val Thr  
130 135 140

Tyr Ser Val Ile Ile Met Ala Leu Ser Arg Leu Lys Met Arg Thr Trp  
145 150 155 160

Lys Lys Trp Glu Thr Glu Thr Ala Ser Leu Glu Tyr Gln Phe Ala Asn  
165 170 175

Asp Pro Ala Arg Phe Arg Phe Thr His Gln Thr Ser Phe Val Lys Arg  
180 185 190

His Leu Gly Leu Ser Ser Thr Pro Gly Val Arg Trp Val Val Ala Phe  
195 200 205

Phe Arg Gln Phe Phe Arg Ser Val Thr Lys Val Asp Tyr Leu Thr Leu  
210 215 220

Arg Ala Gly Phe Ile Asn Ala His Leu Ser His Asn Ser Lys Phe Asp  
225 230 235 240

Phe His Lys Tyr Ile Lys Arg Ser Met Glu Asp Asp Phe Lys Val Val  
245 250 255

Val Gly Ile Ser Leu Pro Leu Trp Cys Val Ala Ile Leu Thr Leu Phe  
260 265 270

Leu Asp Ile Asp Gly Ile Gly Thr Leu Thr Trp Ile Ser Phe Ile Pro  
275 280 285

- 6 -

Leu Val Ile Leu Leu Cys Val Gly Thr Lys Leu Glu Met Ile Ile Met  
290 295 300

Glu Met Ala Leu Glu Ile Gln Asp Arg Ala Ser Val Ile Lys Gly Ala  
305 310 315 320

Pro Val Val Glu Pro Ser Asn Lys Phe Phe Trp Phe His Arg Pro Asp  
325 330 335

Trp Val Leu Phe Phe Ile His Leu Thr Leu Phe Gln Asn Ala Phe Gln  
340 345 350

Met Ala His Phe Val Trp Thr Val Ala Thr Pro Gly Leu Lys Lys Cys  
355 360 365

Phe His Met His Ile Gly Leu Ser Ile Met Lys Val Val Leu Gly Leu  
370 375 380

Ala Leu Gln Phe Leu Cys Ser Tyr Ile Thr Phe Pro Leu Tyr Ala Leu  
385 390 395 400

Val Thr Gln Met Gly Ser Asn Met Lys Arg Ser Ile Phe Asp Glu Gln  
405 410 415

Thr Ala Lys Ala Leu Thr Asn Trp Arg Asn Thr Ala Lys Glu Lys Lys  
420 425 430

Lys Val Arg Asp Thr Asp Met Leu Met Ala Gln Met Ile Gly Asp Ala  
435 440 445

Thr Pro Ser Arg Gly Ala Ser Pro Met Pro Ser Arg Gly Ser Ser Pro  
450 455 460

Val His Leu Leu His Lys Gly Met Gly Arg Ser Asp Asp Pro Gln Ser  
465 470 475 480

Thr Pro Thr Ser Pro Arg Ala Met Glu Glu Ala Arg Asp Met Tyr Pro  
485 490 495

Val Val Val Ala His Pro Val His Arg Leu Asn Pro Ala Asp Arg Arg  
500 505 510

Arg Ser Val Ser Ser Ser Ala Leu Asp Val Asp Ile Pro Ser Ala Asp  
515 520 525

Phe Ser Phe Ser Gln Gly  
530

&lt;210&gt; 5

&lt;211&gt; 1693

&lt;212&gt; DNA

&lt;213&gt; Triticum sp.

&lt;220&gt;

<221> CDS

<222> (1)..(1602)

<220>

<221> misc\_feature

<222> (190) .. (228)

<223> location of the amino acid sequence set forth in  
SEQ ID No:1

<220>

<221> misc\_feature

<222> (1177) .. (1218)

<223> location of the amino acid sequence set forth in  
SEQ ID No:2

<400> 5

atg gcg gag gac tac gag tac ccc ccg gcg cg<sup>g</sup> acg ctg ccg gag acg  
 Met Ala Glu Asp Tyr Glu Tyr Pro Pro Ala Arg Thr Leu Pro Glu Thr  
           1              5              10              15

48

ccg tcc tgg gcg gtg gcg ctc gtc ttc gcc gtc atg atc atc atc gtg tcc  
 Pro Ser Trp Ala Val Ala Leu Val Phe Ala Val Met Ile Ile Val Ser  
 20 25 30

96

gtc ctc ctg gag cac ggc ctc cac aag ctc ggc cat tgg ttc cac aag  
Val Leu Leu Glu His Ala Leu His Lys Leu Gly His Trp Phe His Lys  
35 40 45

144

cgg cac aag aac gcg ctg gcg gag gcg ctg gag aag atc aaa gcg gag  
Arg His Lys Asn Ala Leu Ala Glu Ala Leu Glu Lys Ile Lys Ala Glu  
50 55 60

192

ctg atg ctg gtg ggg ttc atc tcg ctg ctc gcc gtg acg cag gac  
Leu Met Leu Val Gly Phe Ile Ser Leu Leu Leu Ala Val Thr Gln Asp  
65 70 75 80

240

cca atc tcc ggg ata tgc atc tcc gag aag gcc gcc agc atc atg cgg  
 Pro Ile Ser Gly Ile Cys Ile Ser Glu Lys Ala Ala Ser Ile Met Arg  
 85 90 95

288

ccc tgc agc ctg ccc cct ggt tcc gtc aag agc aag tac aaa gac tac  
 Pro Cys Ser Leu Pro Pro Gly Ser Val Lys Ser Lys Tyr Lys Asp Tyr  
 120 105 110

336

tac tgc gcc aaa aag ggc aag gtg tcg cta atg tcc acg ggc agc ttg  
 Tyr Cys Ala Lys Lys Gly Lys Val Ser Leu Met Ser Thr Gly Ser Leu  
 115 120 125

384

cac cag ctc cac atg ttc atc ttc gtg ctc gcc gtc ttc cat gtc acc  
 His Gln Leu His Met Phe Ile Phe Val Leu Ala Val Phe His Val Thr

132

tac agc gtc atc atc atg gct cta agc cgt ctc aaa atg agg aca tgg  
 Tyr Ser Val Ile Ile Met Ala Leu Ser Arg Leu Lys Met Arg Thr Try

- 8 -

aag aaa tgg gag aca gag acc gyc tcc ttg gaa tac cag ttc gca aat	528		
Lys Lys Trp Glu Thr Glu Thr Xaa Ser Leu Glu Tyr Gln Phe Ala Asn			
165	170	175	
gat cct gcg cgg ttc cgc ttc acg cac cag acg tcg ttc gtg aag cgt	576		
Asp Pro Ala Arg Phe Arg Phe Thr His Gln Thr Ser Phe Val Lys Arg			
180	185	190	
cac ctg ggc ctc tcc agc acc ccc ggc atc aga tgg gtg gtg gcc ttc	624		
His Leu Gly Leu Ser Ser Thr Pro Gly Ile Arg Trp Val Val Ala Phe			
195	200	205	
ttc agg cag ttc ttc agg tcg gtc acc aag gtg gac tac ctc acc ctg	672		
Phe Arg Gln Phe Phe Arg Ser Val Thr Lys Val Asp Tyr Leu Thr Leu			
210	215	220	
agg gca ggc ttc atc aac gcg cat ttg tcg cat aac agc aag ttc gac	720		
Arg Ala Gly Phe Ile Asn Ala His Leu Ser His Asn Ser Lys Phe Asp			
225	230	235	240
ttc cac aag tac atc aag agg tcc atg gag gac gac ttc aaa gtc gtc	768		
Phe His Lys Tyr Ile Lys Arg Ser Met Glu Asp Asp Phe Lys Val Val			
245	250	255	
gtt ggc atc agc ctc ccg ctg tgg tgt gtg gcg atc ctc acc ctc ttc	816		
Val Gly Ile Ser Leu Pro Leu Trp Cys Val Ala Ile Leu Thr Leu Phe			
260	265	270	
ctt gat att gac ggg atc ggc acg ctc acc tgg att tct ttc atc cct	864		
Leu Asp Ile Asp Gly Ile Gly Thr Leu Thr Trp Ile Ser Phe Ile Pro			
275	280	285	
ctc gtc atc ctc ttg tgt gtt gga acc aag ctg gag atg atc atc atg	912		
Leu Val Ile Leu Leu Cys Val Gly Thr Lys Leu Glu Met Ile Ile Met			
290	295	300	
gag atg gcc ctg gag atc cag gac cgg gcg agc gtc atc aag ggg gcg	960		
Glu Met Ala Leu Glu Ile Gln Asp Arg Ala Ser Val Ile Lys Gly Ala			
305	310	315	320
ccc gtg gtt gag ccc agc aac aag ttc ttc tgg ttc cac cgc ccc gac	1008		
Pro Val Val Glu Pro Ser Asn Lys Phe Phe Trp Phe His Arg Pro Asp			
325	330	335	
tgg gtc ctc ttc ata cac ctg acg ctg ttc cag aat gcg ttt cag	1056		
Trp Val Leu Phe Phe Ile His Leu Thr Leu Phe Gln Asn Ala Phe Gln			
340	345	350	
atg gca cat ttc gtc tgg aca gtg gcc acg ccc ggc ttg aag aaa tgc	1104		
Met Ala His Phe Val Trp Thr Val Ala Thr Pro Gly Leu Lys Lys Cys			
355	360	365	
ttc cat atg cac atc ggt ctg agc atc atg aag gtc gtg ctg ggg ctg	1152		
Phe His Met His Ile Gly Leu Ser Ile Met Lys Val Val Leu Gly Leu			

- 9 -

370	375	380	
gct ctt cag ttc ctc tgc agc tat atc acc ttc ccc ctc tac gcg ctc Ala Leu Gln Phe Leu Cys Ser Tyr Ile Thr Phe Pro Leu Tyr Ala Leu 385 390 395 400			1200
gtc aca cag atg gga tcg aac atg aag agg tcc atc ttc gac gag cag Val Thr Gln Met Gly Ser Asn Met Lys Arg Ser Ile Phe Asp Glu Gln 405 410 415			1248
acg gcc aag gcg ctg acc aac tgg cgg aac acg gcc aag gag aag aag Thr Ala Lys Ala Leu Thr Asn Trp Arg Asn Thr Ala Lys Glu Lys Lys 420 425 430			1296
aag gtc cga gac acg gac atg ctg atg gcg cag atg atc ggc gac gcg Lys Val Arg Asp Thr Asp Met Leu Met Ala Gln Met Ile Gly Asp Ala 435 440 445			1344
acg ccc agc cga ggc acg tcg ccg atg cct agc cgg gct tcg tca ccg Thr Pro Ser Arg Gly Thr Ser Pro Met Pro Ser Arg Ala Ser Ser Pro 450 455 460			1392
gtg cac ctg ctt cac aag ggc atg gga cgg tcc gac gat ccc cag agc Val His Leu Leu His Lys Gly Met Gly Arg Ser Asp Asp Pro Gln Ser 465 470 475 480			1440
gcg ccg acc tcg cca agg acc atg gag gag gct agg gac atg tac ccg Ala Pro Thr Ser Pro Arg Thr Met Glu Glu Ala Arg Asp Met Tyr Pro 485 490 495			1488
gtt gtg gtg gcg cat ccc gtg cac aga cta aat cct gct gac agg cgg Val Val Val Ala His Pro Val His Arg Leu Asn Pro Ala Asp Arg Arg 500 505 510			1536
agg tcg gtc tct tcg gca ctc gat gcc gac atc ccc agc gca gat Arg Ser Val Ser Ser Ala Leu Asp Ala Asp Ile Pro Ser Ala Asp 515 520 525			1584
ttt tcc ttc agc cag gga tgagacaagt ttctgtattt atgttagtcc Phe Ser Phe Ser Gln Gly 530			1632
aatgtatacg caacatagga tgtcatgatt cgtacaataa gaaataaaaa tttttactga g			1692 1693
<210> 6 <211> 534 <212> PRT <213> Triticum sp.			
<400> 6 Met Ala Glu Asp Tyr Glu Tyr Pro Pro Ala Arg Thr Leu Pro Glu Thr 1 5 10 15			

- 10 -

Pro Ser Trp Ala Val Ala Leu Val Phe Ala Val Met Ile Ile Val Ser  
20 25 30

Val Leu Leu Glu His Ala Leu His Lys Leu Gly His Trp Phe His Lys  
35 40 45

Arg His Lys Asn Ala Leu Ala Glu Ala Leu Glu Lys Ile Lys Ala Glu  
50 55 60

Leu Met Leu Val Gly Phe Ile Ser Leu Leu Ala Val Thr Gln Asp  
65 70 75 80

Pro Ile Ser Gly Ile Cys Ile Ser Glu Lys Ala Ala Ser Ile Met Arg  
85 90 95

Pro Cys Ser Leu Pro Pro Gly Ser Val Lys Ser Lys Tyr Lys Asp Tyr  
100 105 110

Tyr Cys Ala Lys Lys Gly Lys Val Ser Leu Met Ser Thr Gly Ser Leu  
115 120 125

His Gln Leu His Met Phe Ile Phe Val Leu Ala Val Phe His Val Thr  
130 135 140

Tyr Ser Val Ile Ile Met Ala Leu Ser Arg Leu Lys Met Arg Thr Trp  
145 150 155 160

Lys Lys Trp Glu Thr Glu Thr Xaa Ser Leu Glu Tyr Gln Phe Ala Asn  
165 170 175

Asp Pro Ala Arg Phe Arg Phe Thr His Gln Thr Ser Phe Val Lys Arg  
180 185 190

His Leu Gly Leu Ser Ser Thr Pro Gly Ile Arg Trp Val Val Ala Phe  
195 200 205

Phe Arg Gln Phe Phe Arg Ser Val Thr Lys Val Asp Tyr Leu Thr Leu  
210 215 220

Arg Ala Gly Phe Ile Asn Ala His Leu Ser His Asn Ser Lys Phe Asp  
225 230 235 240

Phe His Lys Tyr Ile Lys Arg Ser Met Glu Asp Asp Phe Lys Val Val  
245 250 255

Val Gly Ile Ser Leu Pro Leu Trp Cys Val Ala Ile Leu Thr Leu Phe  
260 265 270

Leu Asp Ile Asp Gly Ile Gly Thr Leu Thr Trp Ile Ser Phe Ile Pro  
275 280 285

Leu Val Ile Leu Leu Cys Val Gly Thr Lys Leu Glu Met Ile Ile Met  
290 295 300

- 11 -

Glu Met Ala Leu Glu Ile Gln Asp Arg Ala Ser Val Ile Lys Gly Ala  
305 310 315 320

Pro Val Val Glu Pro Ser Asn Lys Phe Phe Trp Phe His Arg Pro Asp  
325 330 335

Trp Val Leu Phe Phe Ile His Leu Thr Leu Phe Gln Asn Ala Phe Gln  
340 345 350

Met Ala His Phe Val Trp Thr Val Ala Thr Pro Gly Leu Lys Lys Cys  
355 360 365

Phe His Met His Ile Gly Leu Ser Ile Met Lys Val Val Leu Gly Leu  
370 375 380

Ala Leu Gln Phe Leu Cys Ser Tyr Ile Thr Phe Pro Leu Tyr Ala Leu  
385 390 395 400

Val Thr Gln Met Gly Ser Asn Met Lys Arg Ser Ile Phe Asp Glu Gln  
405 410 415

Thr Ala Lys Ala Leu Thr Asn Trp Arg Asn Thr Ala Lys Glu Lys Lys  
420 425 430

Lys Val Arg Asp Thr Asp Met Leu Met Ala Gln Met Ile Gly Asp Ala  
435 440 445

Thr Pro Ser Arg Gly Thr Ser Pro Met Pro Ser Arg Ala Ser Ser Pro  
450 455 460

Val His Leu Leu His Lys Gly Met Gly Arg Ser Asp Asp Pro Gln Ser  
465 470 475 480

Ala Pro Thr Ser Pro Arg Thr Met Glu Glu Ala Arg Asp Met Tyr Pro  
485 490 495

Val Val Val Ala His Pro Val His Arg Leu Asn Pro Ala Asp Arg Arg  
500 505 510

Arg Ser Val Ser Ser Ser Ala Leu Asp Ala Asp Ile Pro Ser Ala Asp  
515 520 525

Phe Ser Phe Ser Gln Gly  
530

<210> 7  
<211> 1886  
<212> DNA  
<213> Triticum sp.

<220>  
<221> CDS  
<222> (198) .. (1799)

<220>  
 <221> misc\_feature  
 <222> (387)..(425)  
 <223> location of the amino acid sequence set forth in  
 SEQ ID No:1

<220>  
 <221> misc\_feature  
 <222> (1374)..(1415)  
 <223> location of the amino acid sequence set forth in  
 SEQ ID No:2

<400> 7  
 gcagcaacaa gctagacata cctgcgtgcg tacgtacgtt ttcgtttcc tttcttgctc 60  
 cggccggccg gccggccacg tagaatagat acctgcccag gtacgtacct cggtggctca 120  
 gacgatcggc gggtggactt gggtgcgccgc cctgcccgc tccggccaag gaaagagggtt 180  
 gcgctaaaga cgggcgg atg gca aag gac gac ggg tac ccc ccg gcg cgg 230  
 Met Ala Lys Asp Asp Gly Tyr Pro Pro Ala Arg  
 1 5 10  
 acg ctg ccg gag acg ccg tcc tgg gcg gtg gcg ctg gtc ttc gcc gtc 278  
 Thr Leu Pro Glu Thr Pro Ser Trp Ala Val Ala Leu Val Phe Ala Val  
 15 20 25  
 atg atc atc gtc tcc ctc ctg gag cac gcg ctc cac aag ctc ggc 326  
 Met Ile Ile Val Ser Val Leu Leu Glu His Ala Leu His Lys Leu Gly  
 30 35 40  
 cat tgg ttc cac aag cgg cac aag aac gcg ctg gcg gag ggc 374  
 His Trp Phe His Lys Arg His Lys Asn Ala Leu Ala Glu Ala Leu Glu  
 45 50 55  
 aag atg aag gcg gag ctg atg ctg gtg gga ttc atc tcg ctg ctc 422  
 Lys Met Lys Ala Glu Leu Met Leu Val Gly Phe Ile Ser Leu Leu Leu  
 60 65 70 75  
 gcc gtc acg cag gac cca atc tcc ggg ata tgc atc tcc cag aag gcc 470  
 Ala Val Thr Gln Asp Pro Ile Ser Gly Ile Cys Ile Ser Gln Lys Ala  
 80 85 90  
 gcc agc atc atg cgc ccc tgc aag gtg gaa ccc ggt tcc gtc aag agc 518  
 Ala Ser Ile Met Arg Pro Cys Lys Val Glu Pro Gly Ser Val Lys Ser  
 95 100 105  
 aag tac aag gac tac tac tgc gcc aaa gag ggc aag gtg gcg ctc atg 566  
 Lys Tyr Lys Asp Tyr Tyr Cys Ala Lys Glu Gly Lys Val Ala Leu Met  
 110 115 120  
 tcc acg ggc agc ctg cac cag ctc cac ata ttc atc ttc gtg cta gcc 614  
 Ser Thr Gly Ser Leu His Gln Leu His Ile Phe Ile Phe Val Leu Ala  
 125 130 135

gtc ttc cat gtc acc tac agc gtc atc atc atg gct cta agc cgt ctc Val Phe His Val Thr Tyr Ser Val Ile Ile Met Ala Leu Ser Arg Leu 140 145 150 155	662
aag atg aga aca tgg aag aaa tgg gag aca gaa acc gcc tcc ttg gaa Lys Met Arg Thr Trp Lys Lys Trp Glu Thr Glu Thr Ala Ser Leu Glu 160 165 170	710
tac cag ttc gca aat gat cct gcg cgg ttc cgc ttc acg cac cag acg Tyr Gln Phe Ala Asn Asp Pro Ala Arg Phe Arg Phe Thr His Gln Thr 175 180 185	758
tcg ttc gtg aag cgg cac ctg ggc ctg tcc agc acc ccc ggc gtc aga Ser Phe Val Lys Arg His Leu Gly Leu Ser Ser Thr Pro Gly Val Arg 190 195 200	806
tgg gtg gtg gcc ttc ttc agg cag ttc ttc agg tcg gtc acc aag gtg Trp Val Val Ala Phe Phe Arg Gln Phe Phe Arg Ser Val Thr Lys Val 205 210 215	854
gac tac ctc acc ttg agg gca ggc ttc atc aac gcg cac ttg tcg cag Asp Tyr Leu Thr Leu Arg Ala Gly Phe Ile Asn Ala His Leu Ser Gln 220 225 230 235	902
aac agc aag ttc gac ttc cac aag tac atc aag agg tcc atg gag gac Asn Ser Lys Phe Asp Phe His Lys Tyr Ile Lys Arg Ser Met Glu Asp 240 245 250	950
gac ttc aaa gtc gtc gtt ggc atc agc ctc ccg ctg tgg gct gtg gcg Asp Phe Lys Val Val Val Gly Ile Ser Leu Pro Leu Trp Ala Val Ala 255 260 265	998
atc ctc acc ctc ttc ctt gat atc gac ggg atc ggc aca ctc acc tgg Ile Leu Thr Leu Phe Leu Asp Ile Asp Gly Ile Gly Thr Leu Thr Trp 270 275 280	1046
gtt tct ttc atc cct ctc atc atc ctc ttg tgt gtt gga acc aag cta Val Ser Phe Ile Pro Leu Ile Ile Leu Cys Val Gly Thr Lys Leu 285 290 295	1094
gag atg atc atc atg ggg atg gcc ctg gag atc cag gac cgg tcg agc Glu Met Ile Ile Met Gly Met Ala Leu Glu Ile Gln Asp Arg Ser Ser 300 305 310 315	1142
gtc atc aag ggg gca ccc gtg gtc gag ccc agc aac aag ttc ttc tgg Val Ile Lys Gly Ala Pro Val Val Glu Pro Ser Asn Lys Phe Phe Trp 320 325 330	1190
ttc cac cgc ccc gac tgg gtc ctc ttc atc cac ctg acg ctg ttc Phe His Arg Pro Asp Trp Val Leu Phe Phe Ile His Leu Thr Leu Phe 335 340 345	1238
cag aac gcg ttt cag atg gca cat ttc gtg tgg aca gtg gcc acg ccc Gln Asn Ala Phe Gln Met Ala His Phe Val Trp Thr Val Ala Thr Pro 350 355 360	1286

- 14 -

ggc ttg aag gac tgc ttc cat atg aac atc ggg ctg agc atc atc aag Gly Leu Lys Asp Cys Phe His Met Asn Ile Gly Leu Ser Ile Met Lys	365	370	375	1334
gtc gtg ctg ggg ctg gct ctc cag ttc ctg tgc agc tac atc acc ttc Val Val Leu Gly Leu Ala Leu Gln Phe Leu Cys Ser Tyr Ile Thr Phe	380	385	390	395
ccc ctc tac gcg cta gtc aca cag atg gga tca aac atg aag agg tcc Pro Leu Tyr Ala Leu Val Thr Gln Met Gly Ser Asn Met Lys Arg Ser	400	405	410	1430
atc ttc gac gag cag aca gcc aag gcg ctg acc aac tgg cg <sup>g</sup> aac acg Ile Phe Asp Glu Gln Thr Ala Lys Ala Leu Thr Asn Trp Arg Asn Thr	415	420	425	1478
gcc aag gag aag aag gtc cga gac acg gac atg ctg atg gcg cag Ala Lys Glu Lys Lys Val Arg Asp Thr Asp Met Leu Met Ala Gln	430	435	440	1526
atg atc ggc gac gca aca ccc agc cga ggc acg tcc ccg atg cct agc Met Ile Gly Asp Ala Thr Pro Ser Arg Gly Thr Ser Pro Met Pro Ser	445	450	455	1574
cg <sup>g</sup> ggc tca tcg ccg gtg cac ctg ctt cag aag ggc atg gga cg <sup>g</sup> tct Arg Gly Ser Ser Pro Val His Leu Leu Gln Lys Gly Met Gly Arg Ser	460	465	470	475
gac gat ccc cag agc gca ccg acc tcg cca agg acc atg gag gag gct Asp Asp Pro Gln Ser Ala Pro Thr Ser Pro Arg Thr Met Glu Glu Ala	480	485	490	1670
agg gac atg tac ccg gtt gtg gtg gcg cat cct gta cac aga cta aat Arg Asp Met Tyr Pro Val Val Val Ala His Pro Val His Arg Leu Asn	495	500	505	1718
cct gct gac agg cg <sup>g</sup> agg tcg gtc tct tca tca gcc ctc gat gcc gac Pro Ala Asp Arg Arg Ser Val Ser Ser Ala Leu Asp Ala Asp	510	515	520	1766
atc ccc agc gca gat ttt tcc ttc agc cag gga tgagacaagt ttctgtattg Ile Pro Ser Ala Asp Phe Ser Phe Ser Gln Gly	525	530		1819
atgttagtcc aatgtatagc caacatagga t <sup>t</sup> gtatgatt cgtacaataa gaaatacaat				1879
tttttac				1886

<210> 8  
<211> 534  
<212> PRT  
<213> *Triticum* sp.

&lt;400&gt; 8

Met Ala Lys Asp Asp Gly Tyr Pro Pro Ala Arg Thr Leu Pro Glu Thr  
1 5 10 15

Pro Ser Trp Ala Val Ala Leu Val Phe Ala Val Met Ile Ile Val Ser  
20 25 30

Val Leu Leu Glu His Ala Leu His Lys Leu Gly His Trp Phe His Lys  
35 40 45

Arg His Lys Asn Ala Leu Ala Glu Ala Leu Glu Lys Met Lys Ala Glu  
50 55 60

Leu Met Leu Val Gly Phe Ile Ser Leu Leu Ala Val Thr Gln Asp  
65 70 75 80

Pro Ile Ser Gly Ile Cys Ile Ser Gln Lys Ala Ala Ser Ile Met Arg  
85 90 95

Pro Cys Lys Val Glu Pro Gly Ser Val Lys Ser Lys Tyr Lys Asp Tyr  
100 105 110

Tyr Cys Ala Lys Glu Gly Lys Val Ala Leu Met Ser Thr Gly Ser Leu  
115 120 125

His Gln Leu His Ile Phe Ile Phe Val Leu Ala Val Phe His Val Thr  
130 135 140

Tyr Ser Val Ile Ile Met Ala Leu Ser Arg Leu Lys Met Arg Thr Trp  
145 150 155 160

Lys Lys Trp Glu Thr Glu Thr Ala Ser Leu Glu Tyr Gln Phe Ala Asn  
165 170 175

Asp Pro Ala Arg Phe Arg Phe Thr His Gln Thr Ser Phe Val Lys Arg  
180 185 190

His Leu Gly Leu Ser Ser Thr Pro Gly Val Arg Trp Val Val Ala Phe  
195 200 205

Phe Arg Gln Phe Phe Arg Ser Val Thr Lys Val Asp Tyr Leu Thr Leu  
210 215 220

Arg Ala Gly Phe Ile Asn Ala His Leu Ser Gln Asn Ser Lys Phe Asp  
225 230 235 240

Phe His Lys Tyr Ile Lys Arg Ser Met Glu Asp Asp Phe Lys Val Val  
245 250 255

Val Gly Ile Ser Leu Pro Leu Trp Ala Val Ala Ile Leu Thr Leu Phe  
260 265 270

Leu Asp Ile Asp Gly Ile Gly Thr Leu Thr Trp Val Ser Phe Ile Pro  
275 280 285

- 16 -

Leu Ile Ile Leu Leu Cys Val Gly Thr Lys Leu Glu Met Ile Ile Met  
290 295 300

Gly Met Ala Leu Glu Ile Gln Asp Arg Ser Ser Val Ile Lys Gly Ala  
305 310 315 320

Pro Val Val Glu Pro Ser Asn Lys Phe Phe Trp Phe His Arg Pro Asp  
325 330 335

Trp Val Leu Phe Phe Ile His Leu Thr Leu Phe Gln Asn Ala Phe Gln  
340 345 350

Met Ala His Phe Val Trp Thr Val Ala Thr Pro Gly Leu Lys Asp Cys  
355 360 365

Phe His Met Asn Ile Gly Leu Ser Ile Met Lys Val Val Leu Gly Leu  
370 375 380

Ala Leu Gln Phe Leu Cys Ser Tyr Ile Thr Phe Pro Leu Tyr Ala Leu  
385 390 395 400

Val Thr Gln Met Gly Ser Asn Met Lys Arg Ser Ile Phe Asp Glu Gln  
405 410 415

Thr Ala Lys Ala Leu Thr Asn Trp Arg Asn Thr Ala Lys Glu Lys Lys  
420 425 430

Lys Val Arg Asp Thr Asp Met Leu Met Ala Gln Met Ile Gly Asp Ala  
435 440 445

Thr Pro Ser Arg Gly Thr Ser Pro Met Pro Ser Arg Gly Ser Ser Pro  
450 455 460

Val His Leu Leu Gln Lys Gly Met Gly Arg Ser Asp Asp Pro Gln Ser  
465 470 475 480

Ala Pro Thr Ser Pro Arg Thr Met Glu Glu Ala Arg Asp Met Tyr Pro  
485 490 495

Val Val Val Ala His Pro Val His Arg Leu Asn Pro Ala Asp Arg Arg  
500 505 510

Arg Ser Val Ser Ser Ser Ala Leu Asp Ala Asp Ile Pro Ser Ala Asp  
515 520 525

Phe Ser Phe Ser Gln Gly  
530

<210> 9

<211> 2197

<212> DNA

<213> *Arabidopsis thaliana*

<220>

<221> CDS  
<222> (331)..(2037)

<220>  
<221> misc\_feature  
<222> (589)..(627)  
<223> location of the amino acid sequence set forth in  
SEQ ID No:1

<220>  
<221> misc\_feature  
<222> (1603)..(1644)  
<223> location of the amino acid sequence set forth in  
SEQ ID No:2

<400> 9  
agtaatttag ctgttcttct acccctctga tctctcacag gggatcaa at agttttgata 60  
catagagcca caacagtgc attagtgtgt tggtgactac tgtaagggtt gggtttgaa 120  
aagagacatg aaggagtgtt attaggttga ttgtcttcaa gtacctccag tgtcaa acaa 180  
acattgacga ttgattctct tcccataatt tattgttatg cattacatat cacagtaaac 240  
ggactttcaa gtcaacaccg catttatgg ccctcttcat tgtttacgt acgtaatcaa 300  
ggaccaaggg attttgttct tttggctacc atg gcc aca aga tgc ttt tgg tgt 354  
Met Ala Thr Arg Cys Phe Trp Cys  
1 5

tgg acc act ttg ctc ttc tgc tct cag ctg ctt acc ggc ttt gcc cga 402  
Trp Thr Thr Leu Leu Phe Cys Ser Gln Leu Leu Thr Gly Phe Ala Arg  
10 15 20

gct tcc tct gca ggc ggc aaa gag aaa gga ctc tcc caa act ccc 450  
Ala Ser Ser Ala Gly Gly Ala Lys Glu Lys Gly Leu Ser Gln Thr Pro  
25 30 35 40

acc tgg gcc gtt gcc ctc gtc tgt acc ttt ttc att ctt gtc tcc gtc 498  
Thr Trp Ala Val Ala Leu Val Cys Thr Phe Phe Ile Leu Val Ser Val  
45 50 55

ctt ctc gag aag gct ctt cac aga gtt gcc acg tgg ttg tgg gag aaa 546  
Leu Leu Glu Lys Ala Leu His Arg Val Ala Thr Trp Leu Trp Glu Lys  
60 65 70

cat aag aac tct ctg ctt gaa gcc ttg gaa aaa ata aag gcc gag ctg 594  
His Lys Asn Ser Leu Leu Glu Ala Leu Glu Lys Ile Lys Ala Glu Leu  
75 80 85

atg att cta gga ttc att tcc ttg ttg cta acc ttc gga gag cag tac 642  
Met Ile Leu Gly Phe Ile Ser Leu Leu Leu Thr Phe Gly Glu Gln Tyr  
90 95 100

att ctc aag att tgt att cct gaa aag gct gca gcc tct atg tta cct 690

Ile Leu Lys Ile Cys Ile Pro Glu Lys Ala Ala Ala Ser Met Leu Pro			
105	110	115	120
tgt cca gct cct tct act cat gac caa gac aag acc cac cgc aga cgt			738
Cys Pro Ala Pro Ser Thr His Asp Gln Asp Lys Thr His Arg Arg Arg			
125	130	135	
cta gct gct acg acc tct tcc cgc tgc gat gag ggt cat gaa cca			786
Leu Ala Ala Ala Thr Thr Ser Ser Arg Cys Asp Glu Gly His Glu Pro			
140	145	150	
ctc ata cct gcc acg ggt ttg cac cag cta cac att cta ttg ttc ttc			834
Leu Ile Pro Ala Thr Gly Leu His Gln Leu His Ile Leu Leu Phe Phe			
155	160	165	
atg gct gcc ttt cat atc ctc tac agt ttc atc acc atg atg ctt ggc			882
Met Ala Ala Phe His Ile Leu Tyr Ser Phe Ile Thr Met Met Leu Gly			
170	175	180	
aga ctc aag atc cgt ggc tgg aaa aag tgg gag cag gag aca tgt tct			930
Arg Leu Lys Ile Arg Gly Trp Lys Lys Trp Glu Gln Glu Thr Cys Ser			
185	190	195	200
cat gat tac gag ttt tca atc gat cca tca aga ttc aga ctc act cat			978
His Asp Tyr Glu Phe Ser Ile Asp Pro Ser Arg Phe Arg Leu Thr His			
205	210	215	
gag acg tcc ttt gtt aga caa cat tcc agt ttc tgg aca aaa atc ccc			1026
Glu Thr Ser Phe Val Arg Gln His Ser Ser Phe Trp Thr Lys Ile Pro			
220	225	230	
ttc ttc ttt tat gct ggg tgc ttc cta cag cag ttt ttc cga tct gtc			1074
Phe Phe Phe Tyr Ala Gly Cys Phe Leu Gln Gln Phe Phe Arg Ser Val			
235	240	245	
ggg agg act gac tac tta act ctg cgc cat ggc ttc atc gct gcc cat			1122
Gly Arg Thr Asp Tyr Leu Thr Leu Arg His Gly Phe Ile Ala Ala His			
250	255	260	
tta gct cca gga aga aag ttc gac ttc cag aag tat atc aaa aga tca			1170
Leu Ala Pro Gly Arg Lys Phe Asp Phe Gln Lys Tyr Ile Lys Arg Ser			
265	270	275	280
ttg gaa gac gat ttc aag gtg gta gtt gga ata agt cct ctt ttg tgg			1218
Leu Glu Asp Asp Phe Lys Val Val Val Gly Ile Ser Pro Leu Leu Trp			
285	290	295	
gca tca ttt gta att ttc cta ctt ctg aat gtt aat ggc tgg gaa gca			1266
Ala Ser Phe Val Ile Phe Leu Leu Leu Asn Val Asn Gly Trp Glu Ala			
300	305	310	
ttg ttt tgg gcg tca atc cta cct gta ctt atc att cta gct gtc agt			1314
Leu Phe Trp Ala Ser Ile Leu Pro Val Leu Ile Ile Leu Ala Val Ser			
315	320	325	

- 19 -

acg aag ctt caa gcg atc cta aca aga atg gct ctg gga atc acg gag	1362
Thr Lys Leu Gln Ala Ile Leu Thr Arg Met Ala Leu Gly Ile Thr Glu	
330 335 340	
aga cac gca gtt gtt caa ggg ata cct ctc gtg cat ggt tca gat aag	1410
Arg His Ala Val Val Gln Gly Ile Pro Leu Val His Gly Ser Asp Lys	
345 350 355 360	
tac ttt tgg ttt aat cgc cct cag ttg cta ctt cat ctt ctt cac ttc	1458
Tyr Phe Trp Phe Asn Arg Pro Gln Leu Leu His Leu Leu His Phe	
365 370 375	
gcc tta ttt cag aat gct ttc cag cta aca tac ttc ttc tgg gtc tgg	1506
Ala Leu Phe Gln Asn Ala Phe Gln Leu Thr Tyr Phe Trp Val Trp	
380 385 390	
tat tcc ttt ggg cta aaa tct tgc ttt cac acg gat ttc aaa cta gtc	1554
Tyr Ser Phe Gly Leu Lys Ser Cys Phe His Thr Asp Phe Lys Leu Val	
395 400 405	
atc gta aaa ctc tct cta ggc gtt gga gct ttg att ttg tgc agc tac	1602
Ile Val Lys Leu Ser Leu Gly Val Gly Ala Leu Ile Leu Cys Ser Tyr	
410 415 420	
atc aca ctt cct ttg tat gca cta gtt act cag atg ggt tca aac atg	1650
Ile Thr Leu Pro Leu Tyr Ala Leu Val Thr Gln Met Gly Ser Asn Met	
425 430 435 440	
aag aaa gct gtg ttt gat gag caa atg gca aaa gcg ttg aag aaa tgg	1698
Lys Lys Ala Val Phe Asp Glu Gln Met Ala Lys Ala Leu Lys Lys Trp	
445 450 455	
cac atg act gtg aag aag aaa ggc aaa gcg aga aag cca cca aca	1746
His Met Thr Val Lys Lys Lys Gly Lys Ala Arg Lys Pro Pro Thr	
460 465 470	
gag acc ctt ggt gtt tct gac act gtc agc acc tct acc tca tcc ttt	1794
Glu Thr Leu Gly Val Ser Asp Thr Val Ser Thr Ser Thr Ser Ser Phe	
475 480 485	
cac gcc tct gga gcc act cta ctc cgc tcc aag acc act ggt cac tcg	1842
His Ala Ser Gly Ala Thr Leu Leu Arg Ser Lys Thr Thr Gly His Ser	
490 495 500	
aca gcc tct tat atg agt aat ttc gag gac caa agc atg tct gat ctt	1890
Thr Ala Ser Tyr Met Ser Asn Phe Glu Asp Gln Ser Met Ser Asp Leu	
505 510 515 520	
gaa gct gag cca tta tcc cct gaa cca ata gag ggg cac act ctc gtc	1938
Glu Ala Glu Pro Leu Ser Pro Glu Pro Ile Glu Gly His Thr Leu Val	
525 530 535	
agg gtt ggt gat cag aac aca gag ata gaa tat act gga gat att agt	1986
Arg Val Gly Asp Gln Asn Thr Glu Ile Glu Tyr Thr Gly Asp Ile Ser	
540 545 550	

cct gga aac caa ttc tcc ttt gtg aag aac gtt cct gct aat gat att 2034  
 Pro Gly Asn Gln Phe Ser Phe Val Lys Asn Val Pro Ala Asn Asp Ile  
 555 560 565

gac taatattcaa aatgaatgca gaacaaaatcc atcatccggc ctttattttc 2087  
 Asp

tattacatgt atgccaacaa ttgcttcgcc aagtgttacc aactaggttt tctgtataag 2147

gctgtatccc agagctaaaa aaaaaaaaaa aaaaaaaaaa ctaaattact 2197

<210> 10

<211> 569

<212> PRT

<213> Arabidopsis thaliana

<400> 10

Met	Ala	Thr	Arg	Cys	Phe	Trp	Cys	Trp	Thr	Thr	Leu	Leu	Phe	Cys	Ser
1															15

Gln	Leu	Leu	Thr	Gly	Phe	Ala	Arg	Ala	Ser	Ser	Ala	Gly	Gly	Ala	Lys
	20								25					30	

Glu	Lys	Gly	Leu	Ser	Gln	Thr	Pro	Thr	Trp	Ala	Val	Ala	Leu	Val	Cys
	35								40					45	

Thr	Phe	Phe	Ile	Leu	Val	Ser	Val	Leu	Leu	Glu	Lys	Ala	Leu	His	Arg
	50								55					60	

Val	Ala	Thr	Trp	Leu	Trp	Glu	Lys	His	Lys	Asn	Ser	Leu	Leu	Glu	Ala
65									70					75	80

Leu	Glu	Lys	Ile	Lys	Ala	Glu	Leu	Met	Ile	Leu	Gly	Phe	Ile	Ser	Leu
	85								90					95	

Leu	Leu	Thr	Phe	Gly	Glu	Gln	Tyr	Ile	Leu	Lys	Ile	Cys	Ile	Pro	Glu
	100							105						110	

Lys	Ala	Ala	Ala	Ser	Met	Leu	Pro	Cys	Pro	Ala	Pro	Ser	Thr	His	Asp
	115							120					125		

Gln	Asp	Lys	Thr	His	Arg	Arg	Arg	Leu	Ala	Ala	Thr	Thr	Ser	Ser
130								135					140	

Arg	Cys	Asp	Glu	Gly	His	Glu	Pro	Leu	Ile	Pro	Ala	Thr	Gly	Leu	His
145								150					155		160

Gln	Leu	His	Ile	Leu	Leu	Phe	Phe	Met	Ala	Ala	Phe	His	Ile	Leu	Tyr
	165							170					175		

Ser	Phe	Ile	Thr	Met	Met	Leu	Gly	Arg	Leu	Lys	Ile	Arg	Gly	Trp	Lys
	180							185					190		

- 21 -

Lys Trp Glu Gln Glu Thr Cys Ser His Asp Tyr Glu Phe Ser Ile Asp  
195 200 205

Pro Ser Arg Phe Arg Leu Thr His Glu Thr Ser Phe Val Arg Gln His  
210 215 220

Ser Ser Phe Trp Thr Lys Ile Pro Phe Phe Tyr Ala Gly Cys Phe  
225 230 235 240

Leu Gln Gln Phe Phe Arg Ser Val Gly Arg Thr Asp Tyr Leu Thr Leu  
245 250 255

Arg His Gly Phe Ile Ala Ala His Leu Ala Pro Gly Arg Lys Phe Asp  
260 265 270

Phe Gln Lys Tyr Ile Lys Arg Ser Leu Glu Asp Asp Phe Lys Val Val  
275 280 285

Val Gly Ile Ser Pro Leu Leu Trp Ala Ser Phe Val Ile Phe Leu Leu  
290 295 300

Leu Asn Val Asn Gly Trp Glu Ala Leu Phe Trp Ala Ser Ile Leu Pro  
305 310 315 320

Val Leu Ile Ile Leu Ala Val Ser Thr Lys Leu Gln Ala Ile Leu Thr  
325 330 335

Arg Met Ala Leu Gly Ile Thr Glu Arg His Ala Val Val Gln Gly Ile  
340 345 350

Pro Leu Val His Gly Ser Asp Lys Tyr Phe Trp Phe Asn Arg Pro Gln  
355 360 365

Leu Leu Leu His Leu Leu His Phe Ala Leu Phe Gln Asn Ala Phe Gln  
370 375 380

Leu Thr Tyr Phe Phe Trp Val Trp Tyr Ser Phe Gly Leu Lys Ser Cys  
385 390 395 400

Phe His Thr Asp Phe Lys Leu Val Ile Val Lys Leu Ser Leu Gly Val  
405 410 415

Gly Ala Leu Ile Leu Cys Ser Tyr Ile Thr Leu Pro Leu Tyr Ala Leu  
420 425 430

Val Thr Gln Met Gly Ser Asn Met Lys Lys Ala Val Phe Asp Glu Gln  
435 440 445

Met Ala Lys Ala Leu Lys Lys Trp His Met Thr Val Lys Lys Lys Lys  
450 455 460

Gly Lys Ala Arg Lys Pro Pro Thr Glu Thr Leu Gly Val Ser Asp Thr  
465 470 475 480

Val Ser Thr Ser Thr Ser Ser Phe His Ala Ser Gly Ala Thr Leu Leu

- 22 -

485

490

495

Arg Ser Lys Thr Thr Gly His Ser Thr Ala Ser Tyr Met Ser Asn Phe  
500 505 510

Glu Asp Gln Ser Met Ser Asp Leu Glu Ala Glu Pro Leu Ser Pro Glu  
 515 520 525

Pro Ile Glu Gly His Thr Leu Val Arg Val Gly Asp Gln Asn Thr Glu  
530 535 540

Ile Glu Tyr Thr Gly Asp Ile Ser Pro Gly Asn Gln Phe Ser Phe Val  
545 550 555 560

Lys Asn Val Pro Ala Asn Asp Ile Asp  
565

<210> 11  
<211> 1935  
<212> DNA  
<213> *Arabidopsis thaliana*

<220>  
<221> CDS  
<222> (89) .. (1807)

<220>  
<221> misc\_feature  
<222> (269)..(307)  
<223> location of the amino acid sequence set forth in  
SEQ ID No:1

<220>  
<221> misc\_feature  
<222> (1370)..(1411)  
<223> location of the amino acid sequence set forth in  
SEQ ID No:2

<400> 11 cagtgtgagt aattttagtaa aaagacaaga tctctggtct ggaatttagaa gaatcttatt 60

tgggtttttt tcttaggatt aagctcta atg gca gat caa gta aaa gag cgg 112  
Met Ala Asp Gln Val Lys Glu Arg  
1 5

act tta gag gag acc tct acg tgg gca gtt gct gtt gtt tgc ttt gtc 160  
 Thr Leu Glu Glu Thr Ser Thr Trp Ala Val Ala Val Val Cys Phe Val  
     10                15                20

tta ctc ttt att tcg att gtc ctc gaa cat tct att cac aaa att gga 208  
 Leu Leu Phe Ile Ser Ile Val Leu Glu His Ser Ile His Lys Ile Gly  
 25                   30                   35                   40

acc tgg ttt aaa aag aag cac aag cag gct ctt ttt gaa gct ctt gaa 256

Thr Trp Phe Lys Lys His Lys Gln Ala Leu Phe Glu Ala Leu Glu			
45	50	55	
aag gtc aaa gca gag ctt atg ctg ttg gga ttc ata tca cta cta ctc			304
Lys Val Lys Ala Glu Leu Met Leu Leu Gly Phe Ile Ser Leu Leu Leu			
60	65	70	
aca att gga caa aca cca atc tca aat atc tgc atc tcc cag aaa gtt			352
Thr Ile Gly Gln Thr Pro Ile Ser Asn Ile Cys Ile Ser Gln Lys Val			
75	80	85	
gct tca aca atg cac cct tgc agt gct gct gaa gaa gct aaa aaa tac			400
Ala Ser Thr Met His Pro Cys Ser Ala Ala Glu Glu Ala Lys Lys Tyr			
90	95	100	
ggc aag aaa gac gcc gga aag aaa gat gat gga gat gga gat aaa ccc			448
Gly Lys Lys Asp Ala Gly Lys Lys Asp Asp Gly Asp Gly Asp Lys Pro			
105	110	115	120
ggt cga aga ctt ctt ctt gag tta gct gaa tct tat atc cat aga aga			496
Gly Arg Arg Leu Leu Leu Glu Leu Ala Glu Ser Tyr Ile His Arg Arg			
125	130	135	
agt tta gcc acc aaa ggc tat gac aaa tgt gca gag aag ggg aaa gtg			544
Ser Leu Ala Thr Lys Gly Tyr Asp Lys Cys Ala Glu Lys Gly Lys Val			
140	145	150	
gct ttt gta tct gct tat gga atc cac cag ctg cat ata ttc atc ttc			592
Ala Phe Val Ser Ala Tyr Gly Ile His Gln Leu His Ile Phe Ile Phe			
155	160	165	
gtg ctc gcg gtt gtt cat gtt tac tgc att gtt act tat gct ttc			640
Val Leu Ala Val Val His Val Val Tyr Cys Ile Val Thr Tyr Ala Phe			
170	175	180	
gga aag atc aag atg agg acg tgg aag tcg tgg gag gaa gag aca aag			688
Gly Lys Ile Lys Met Arg Thr Trp Lys Ser Trp Glu Glu Thr Lys			
185	190	195	200
aca ata gag tat cag tat tcc aac gat cct gag agg ttc agg ttt gcg			736
Thr Ile Glu Tyr Gln Tyr Ser Asn Asp Pro Glu Arg Phe Arg Phe Ala			
205	210	215	
agg gac aca tct ttt ggg aga aga cat ctc aat ttc tgg agc aag acg			784
Arg Asp Thr Ser Phe Gly Arg Arg His Leu Asn Phe Trp Ser Lys Thr			
220	225	230	
aga gtc aca cta tgg att gtt tgt ttt aga cag ttc ttt gga tct			832
Arg Val Thr Leu Trp Ile Val Cys Phe Phe Arg Gln Phe Phe Gly Ser			
235	240	245	
gtc acc aaa gtt gat tac tta gca cta aga cat ggt ttc atc atg gcg			880
Val Thr Lys Val Asp Tyr Leu Ala Leu Arg His Gly Phe Ile Met Ala			
250	255	260	

cat ttt gct ccc ggt aac gaa tca aga ttc gat ttc cgc aag tat att His Phe Ala Pro Gly Asn Glu Ser Arg Phe Asp Phe Arg Lys Tyr Ile 265 270 275 280	928
cag aga tca tta gag aaa gac ttc aaa acc gtt gtt gaa atc agt ccg Gln Arg Ser Leu Glu Lys Asp Phe Lys Thr Val Val Glu Ile Ser Pro 285 290 295	976
gtt atc tgg ttt gtc gct gtg cta ttc ctc ttg acc aat tca tat gga Val Ile Trp Phe Val Ala Val Leu Phe Leu Leu Thr Asn Ser Tyr Gly 300 305 310	1024
tta cgt tct tac ctc tgg tta cca ttc att cca cta gtc gta att cta Leu Arg Ser Tyr Leu Trp Leu Pro Phe Ile Pro Leu Val Val Ile Leu 315 320 325	1072
ata gtt gga aca aag ctt gaa gtc ata ata aca aaa ttg ggt cta aga Ile Val Gly Thr Lys Leu Glu Val Ile Ile Thr Lys Leu Gly Leu Arg 330 335 340	1120
atc caa gag aaa ggt gat gtg gtg aga ggc gcc cca gtg gtt cag cct Ile Gln Glu Lys Gly Asp Val Val Arg Gly Ala Pro Val Val Gln Pro 345 350 355 360	1168
ggg gat gac ctc ttc tgg ttt ggc aag cca cgc ttc att ctt ttc ctt Gly Asp Asp Leu Phe Trp Phe Gly Lys Pro Arg Phe Ile Leu Phe Leu 365 370 375	1216
att cac ttg gtc ctt ttt acg aat gca ttt caa ctt gca ttc ttt gcc Ile His Leu Val Leu Phe Thr Asn Ala Phe Gln Leu Ala Phe Phe Ala 380 385 390	1264
tgg agt acg tat gaa ttc aat ctc aat aat tgt ttc cat gaa agc act Trp Ser Thr Tyr Glu Phe Asn Leu Asn Asn Cys Phe His Glu Ser Thr 395 400 405	1312
gca gat gtg gtc att aga ctt gta gtt gga gct gtt gtg cag ata ctt Ala Asp Val Val Ile Arg Leu Val Val Gly Ala Val Val Gln Ile Leu 410 415 420	1360
tgc agc tat gtg act ctt cca ctc tat gca ctt gtt act cag atg ggt Cys Ser Tyr Val Thr Leu Pro Leu Tyr Ala Leu Val Thr Gln Met Gly 425 430 435 440	1408
agt aaa atg aag cca aca gta ttc aac gat aga gta gcc acg gca tta Ser Lys Met Lys Pro Thr Val Phe Asn Asp Arg Val Ala Thr Ala Leu 445 450 455	1456
aag aag tgg cat cac act gca aag aac gag acg aaa cac gga aga cac Lys Lys Trp His His Thr Ala Lys Asn Glu Thr Lys His Gly Arg His 460 465 470	1504
tcg gga tcc aat aca cct ttc tct agc cgt cca act aca cca aca cat Ser Gly Ser Asn Thr Pro Phe Ser Ser Arg Pro Thr Thr Pro Thr His 475 480 485	1552

- 25 -

ggc tca tct cca atc cat ctc ctt cac aat ttc aat aac cggtt Gly Ser Ser Pro Ile His Leu Leu His Asn Phe Asn Asn Arg Ser Val	1600
490 495 500	
gaa aat tac cca agt tct cct cct aga tac tct ggtt cat ggtt cat Glu Asn Tyr Pro Ser Ser Pro Ser Pro Arg Tyr Ser Gly His Gly His	1648
505 510 515 520	
cat gaa cac caa ttt tgg gat cct gag tct caa cac caa gaa gct gaa His Glu His Gln Phe Trp Asp Pro Glu Ser Gln His Gln Glu Ala Glu	1696
525 530 535	
act tcc aca cat cat tct ctt gcgtt cat gaa agc tca gaa cct gtt ctt Thr Ser Thr His His Ser Leu Ala His Glu Ser Ser Glu Pro Val Leu	1744
540 545 550	
gca tct gtg gaa ctt cct cct ata agg act agc aaa agc tta aga gat Ala Ser Val Glu Leu Pro Pro Ile Arg Thr Ser Lys Ser Leu Arg Asp	1792
555 560 565	
ttt tct ttt aag aaa tgatgattct tgtttgctat atttgatttc gtacagtggg Phe Ser Phe Lys Lys	1847
570	
aattttgtca tatgaaaata atttcttgta cattactagt ttgataagaa ataaccatat	1907
ctatatggat acaaaaaaaaaaaaaaa	1935
<210> 12	
<211> 573	
<212> PRT	
<213> Arabidopsis thaliana	
<400> 12	
Met Ala Asp Gln Val Lys Glu Arg Thr Leu Glu Glu Thr Ser Thr Trp	15
1 5 10	
Ala Val Ala Val Val Cys Phe Val Leu Leu Phe Ile Ser Ile Val Leu	30
20 25	
Glu His Ser Ile His Lys Ile Gly Thr Trp Phe Lys Lys His Lys	45
35 40	
Gln Ala Leu Phe Glu Ala Leu Glu Lys Val Lys Ala Glu Leu Met Leu	60
50 55	
Leu Gly Phe Ile Ser Leu Leu Thr Ile Gly Gln Thr Pro Ile Ser	80
65 70 75	
Asn Ile Cys Ile Ser Gln Lys Val Ala Ser Thr Met His Pro Cys Ser	95
85 90	
Ala Ala Glu Glu Ala Lys Lys Tyr Gly Lys Lys Asp Ala Gly Lys	

- 26 -

	100	105	110
Asp Asp Gly Asp Gly Asp Lys Pro Gly Arg Arg Leu Leu Leu Glu Leu			
115	120	125	
Ala Glu Ser Tyr Ile His Arg Arg Ser Leu Ala Thr Lys Gly Tyr Asp			
130	135	140	
Lys Cys Ala Glu Lys Gly Lys Val Ala Phe Val Ser Ala Tyr Gly Ile			
145	150	155	160
His Gln Leu His Ile Phe Ile Phe Val Leu Ala Val Val His Val Val			
165	170	175	
Tyr Cys Ile Val Thr Tyr Ala Phe Gly Lys Ile Lys Met Arg Thr Trp			
180	185	190	
Lys Ser Trp Glu Glu Glu Thr Lys Thr Ile Glu Tyr Gln Tyr Ser Asn			
195	200	205	
Asp Pro Glu Arg Phe Arg Phe Ala Arg Asp Thr Ser Phe Gly Arg Arg			
210	215	220	
His Leu Asn Phe Trp Ser Lys Thr Arg Val Thr Leu Trp Ile Val Cys			
225	230	235	240
Phe Phe Arg Gln Phe Phe Gly Ser Val Thr Lys Val Asp Tyr Leu Ala			
245	250	255	
Leu Arg His Gly Phe Ile Met Ala His Phe Ala Pro Gly Asn Glu Ser			
260	265	270	
Arg Phe Asp Phe Arg Lys Tyr Ile Gln Arg Ser Leu Glu Lys Asp Phe			
275	280	285	
Lys Thr Val Val Glu Ile Ser Pro Val Ile Trp Phe Val Ala Val Leu			
290	295	300	
Phe Leu Leu Thr Asn Ser Tyr Gly Leu Arg Ser Tyr Leu Trp Leu Pro			
305	310	315	320
Phe Ile Pro Leu Val Val Ile Leu Ile Val Gly Thr Lys Leu Glu Val			
325	330	335	
Ile Ile Thr Lys Leu Gly Leu Arg Ile Gln Glu Lys Gly Asp Val Val			
340	345	350	
Arg Gly Ala Pro Val Val Gln Pro Gly Asp Asp Leu Phe Trp Phe Gly			
355	360	365	
Lys Pro Arg Phe Ile Leu Phe Leu Ile His Leu Val Leu Phe Thr Asn			
370	375	380	
Ala Phe Gln Leu Ala Phe Phe Ala Trp Ser Thr Tyr Glu Phe Asn Leu			
385	390	395	400

Asn Asn Cys Phe His Glu Ser Thr Ala Asp Val Val Ile Arg Leu Val  
405 410 415

Val Gly Ala Val Val Gln Ile Leu Cys Ser Tyr Val Thr Leu Pro Leu  
420 425 430

Tyr Ala Leu Val Thr Gln Met Gly Ser Lys Met Lys Pro Thr Val Phe  
435 440 445

Asn Asp Arg Val Ala Thr Ala Leu Lys Lys Trp His His Thr Ala Lys  
450 455 460

Asn Glu Thr Lys His Gly Arg His Ser Gly Ser Asn Thr Pro Phe Ser  
465 470 475 480

Ser Arg Pro Thr Thr Pro Thr His Gly Ser Ser Pro Ile His Leu Leu  
485 490 495

His Asn Phe Asn Asn Arg Ser Val Glu Asn Tyr Pro Ser Ser Pro Ser  
500 505 510

Pro Arg Tyr Ser Gly His Gly His His Glu His Gln Phe Trp Asp Pro  
515 520 525

Glu Ser Gln His Gln Glu Ala Glu Thr Ser Thr His His Ser Leu Ala  
530 535 540

His Glu Ser Ser Glu Pro Val Leu Ala Ser Val Glu Leu Pro Pro Ile  
545 550 555 560

Arg Thr Ser Lys Ser Leu Arg Asp Phe Ser Phe Lys Lys  
565 570

<210> 13  
<211> 1811  
<212> DNA  
<213> Arabidopsis thaliana

<220>  
<221> CDS  
<222> (56)..(1633)

<220>  
<221> misc\_feature  
<222> (236)..(274)  
<223> location of the amino acid sequence set forth in  
SEQ ID No:1

<220>  
<221> misc\_feature  
<222> (1328)..(1369)  
<223> location of the amino acid sequence set forth in  
SEQ ID No:2

<400> 13  
 gttcccagat tcacatcttac ttattgtctta aattctctct ggtgtgagaa gtaaa atg 58  
 Gly His Gly Glu Gly Met Ser Leu Glu Phe Thr Pro Thr Trp Val  
 5 10 15  
 Met 1

ggt cac gga gga gaa ggg atg tcg ctt gaa ttc act ccg acg tgg gtc 106  
 Gly His Gly Glu Gly Met Ser Leu Glu Phe Thr Pro Thr Trp Val  
 5 10 15  
 Val Ala Gly Val Cys Thr Val Ile Val Ala Ile Ser Leu Ala Val Glu  
 20 25 30  
 154

cgt ttg ctt cac tat ttc ggt act gtt ctt aag aag aag aag caa aaa 202  
 Arg Leu Leu His Tyr Phe Gly Thr Val Leu Lys Lys Lys Lys Gln Lys  
 35 40 45  
 250

ccc ctt tac gaa gcc ctt caa aag gtt aaa gaa gag ctg atg ttg tta  
 Pro Leu Tyr Glu Ala Leu Gln Lys Val Lys Glu Leu Met Leu Leu  
 50 55 60 65  
 298

ggg ttt ata tcg ctg tta ctg acg gta ttc caa ggg ctc att tcc aaa  
 Gly Phe Ile Ser Leu Leu Leu Thr Val Phe Gln Gly Leu Ile Ser Lys  
 70 75 80  
 346

tcc tgt gtg aaa gaa aat gtg ctt atg cat atg ctt cca tgt tct ctc  
 Phe Cys Val Lys Glu Asn Val Leu Met His Met Leu Pro Cys Ser Leu  
 85 90 95  
 394

gat tca aga cga gaa gct ggg gca agt gaa cat aaa aac gtt aca gca  
 Asp Ser Arg Arg Glu Ala Gly Ala Ser Glu His Lys Asn Val Thr Ala  
 100 105 110  
 442

aaa gaa cat ttt cag act ttt tta cct att gtt gga acc act agg cgt  
 Lys Glu His Phe Gln Thr Phe Leu Pro Ile Val Gly Thr Thr Arg Arg  
 115 120 125  
 490

cta ctt gct gaa cat gct gct gtg caa gtt ggt tac tgt agc gaa aag  
 Leu Leu Ala Glu His Ala Ala Val Gln Val Gly Tyr Cys Ser Glu Lys  
 130 135 140 145  
 538

ggt aaa gta cca ttg ctt tcg ctt gag gca ttg cac cat cta cat att  
 Gly Lys Val Pro Leu Leu Ser Leu Glu Ala Leu His His Leu His Ile  
 150 155 160  
 586

tcc atc ttc gtc ctc gcc ata tcc cat gtg aca ttc tgt gtc ctt acc  
 Phe Ile Phe Val Leu Ala Ile Ser His Val Thr Phe Cys Val Leu Thr  
 165 170 175  
 634

gtg att ttt gga agc aca agg att cac caa tgg aag aaa tgg gag gat  
 Val Ile Phe Gly Ser Thr Arg Ile His Gln Trp Lys Lys Trp Glu Asp  
 180 185 190  
 682

tcg atc gca gat gag aag ttt gac ccc gaa aca gct ctc agg aaa aga

Ser Ile Ala Asp Glu Lys Phe Asp Pro Glu Thr Ala Leu Arg Lys Arg			
195	200	205	
agg gtc act cat gta cac aac cat gct ttt att aaa gag cat ttt ctt			730
Arg Val Thr His Val His Asn His Ala Phe Ile Lys Glu His Phe Leu			
210	215	220	225
ggt att ggc aaa gat tca gtc atc ctc gga tgg acg caa tcc ttt ctc			778
Gly Ile Gly Lys Asp Ser Val Ile Leu Gly Trp Thr Gln Ser Phe Leu			
230	235	240	
aag caa ttc tat gat tct gtg acg aaa tca gat tac gtg act tta cgt			826
Lys Gln Phe Tyr Asp Ser Val Thr Lys Ser Asp Tyr Val Thr Leu Arg			
245	250	255	
ctt ggt ttc att atg aca cat tgt aag gga aac ccc aag ctt aat ttc			874
Leu Gly Phe Ile Met Thr His Cys Lys Gly Asn Pro Lys Leu Asn Phe			
260	265	270	
cac aag tat atg atg cgc gct yta gag gat gat ttc aaa caa gtt gtt			922
His Lys Tyr Met Met Arg Ala Xaa Glu Asp Asp Phe Lys Gln Val Val			
275	280	285	
ggt att agt tgg tat ctt tgg atc ttt gtc gtc atc ttt ttg ctg cta			970
Gly Ile Ser Trp Tyr Leu Trp Ile Phe Val Val Ile Phe Leu Leu Leu			
290	295	300	305
aat gtt aac gga tgg cac aca tat ttc tgg ata gca ttt att ccc ttt			1018
Asn Val Asn Gly Trp His Thr Tyr Phe Trp Ile Ala Phe Ile Pro Phe			
310	315	320	
gct ttg ctt ctt gct gtg gga aca aag ttg gag cat gtg att gca cag			1066
Ala Leu Leu Ala Val Gly Thr Lys Leu Glu His Val Ile Ala Gln			
325	330	335	
tta gct cat gaa gtt gca gag aaa cat gta gcc att gaa gga gac tta			1114
Leu Ala His Glu Val Ala Glu Lys His Val Ala Ile Glu Gly Asp Leu			
340	345	350	
gtg gtg aaa ccc tca gat gag cat ttc tgg ttc agc aaa cct caa att			1162
Val Val Lys Pro Ser Asp Glu His Phe Trp Phe Ser Lys Pro Gln Ile			
355	360	365	
gtt ctc tac ttg atc cat ttt atc ctc ttc cag aat gct ttt gag att			1210
Val Leu Tyr Leu Ile His Phe Ile Leu Phe Gln Asn Ala Phe Glu Ile			
370	375	380	385
gcg ttt ttc ttt tgg att tgg gtt aca tac ggc ttc gac tcg tgc att			1258
Ala Phe Phe Phe Trp Ile Trp Val Thr Tyr Gly Phe Asp Ser Cys Ile			
390	395	400	
atg gga cag gtg aga tac att gtt cca aga ttg gtt atc ggg gtc ttc			1306
Met Gly Gln Val Arg Tyr Ile Val Pro Arg Leu Val Ile Gly Val Phe			
405	410	415	

- 30 -

att caa gtg ctt tgc agt tac agt aca ctg cct ctt tac gcc atc gtc      1354  
 Ile Gln Val Leu Cys Ser Tyr Ser Thr Leu Pro Leu Tyr Ala Ile Val  
 420                          425                          430

tca cag atg gga agt agc ttc aag aaa gct ata ttc gag gag aat gtg      1402  
 Ser Gln Met Gly Ser Ser Phe Lys Lys Ala Ile Phe Glu Glu Asn Val  
 435                          440                          445

cag gtt ggt ctt gtt ggt tgg gca cag aaa gtg aaa caa aag aga gac      1450  
 Gln Val Gly Leu Val Gly Trp Ala Gln Lys Val Lys Gln Lys Arg Asp  
 450                          455                          460                          465

cta aaa gct gca gct agt aat gga aac gaa gga agc tct cag gct ggt      1498  
 Leu Lys Ala Ala Ser Asn Gly Asn Glu Gly Ser Ser Gln Ala Gly  
 470                          475                          480

cct ggt cct gat tct ggt tct ggt cct gct gct ggt cct ggt      1546  
 Pro Gly Pro Asp Ser Gly Ser Gly Ser Ala Pro Ala Ala Gly Pro Gly  
 485                          490                          495

gca ggt ttt gca gga att cag ctc agc aga gta aca aga aac aac gca      1594  
 Ala Gly Phe Ala Gly Ile Gln Leu Ser Arg Val Thr Arg Asn Asn Ala  
 500                          505                          510

ggg gac aca aac aat gag att aca cct gat cat aac aac tgagcagaga      1643  
 Gly Asp Thr Asn Asn Glu Ile Thr Pro Asp His Asn Asn  
 515                          520                          525

tattatcttt tccatTTAGA ggatcatcat cagATTTAG cttcaaggTC cgGTTTGTG      1703  
 gTTTatacat aagttagt gacttgattt tttgttttg ttacaaagtt accatcttg      1763  
 gattagaatt gggaaattga atctgtttgt aaaaaaaaaa aaaaaaaaaa      1811

<210> 14  
<211> 526  
<212> PRT  
<213> Arabidopsis thaliana

<400> 14  
Met Gly His Gly Gly Glu Gly Met Ser Leu Glu Phe Thr Pro Thr Trp  
 1                          5                          10                          15

Val Val Ala Gly Val Cys Thr Val Ile Val Ala Ile Ser Leu Ala Val  
 20                          25                          30

Glu Arg Leu Leu His Tyr Phe Gly Thr Val Leu Lys Lys Lys Lys Gln  
 35                          40                          45

Lys Pro Leu Tyr Glu Ala Leu Gln Lys Val Lys Glu Glu Leu Met Leu  
 50                          55                          60

Leu Gly Phe Ile Ser Leu Leu Leu Thr Val Phe Gln Gly Leu Ile Ser  
 65                          70                          75                          80

Lys Phe Cys Val Lys Glu Asn Val Leu Met His Met Leu Pro Cys Ser  
85 90 95

Leu Asp Ser Arg Arg Glu Ala Gly Ala Ser Glu His Lys Asn Val Thr  
100 105 110

Ala Lys Glu His Phe Gln Thr Phe Leu Pro Ile Val Gly Thr Thr Arg  
115 120 125

Arg Leu Leu Ala Glu His Ala Ala Val Gln Val Gly Tyr Cys Ser Glu  
130 135 140

Lys Gly Lys Val Pro Leu Leu Ser Leu Glu Ala Leu His His Leu His  
145 150 155 160

Ile Phe Ile Phe Val Leu Ala Ile Ser His Val Thr Phe Cys Val Leu  
165 170 175

Thr Val Ile Phe Gly Ser Thr Arg Ile His Gln Trp Lys Lys Trp Glu  
180 185 190

Asp Ser Ile Ala Asp Glu Lys Phe Asp Pro Glu Thr Ala Leu Arg Lys  
195 200 205

Arg Arg Val Thr His Val His Asn His Ala Phe Ile Lys Glu His Phe  
210 215 220

Leu Gly Ile Gly Lys Asp Ser Val Ile Leu Gly Trp Thr Gln Ser Phe  
225 230 235 240

Leu Lys Gln Phe Tyr Asp Ser Val Thr Lys Ser Asp Tyr Val Thr Leu  
245 250 255

Arg Leu Gly Phe Ile Met Thr His Cys Lys Gly Asn Pro Lys Leu Asn  
260 265 270

Phe His Lys Tyr Met Met Arg Ala Xaa Glu Asp Asp Phe Lys Gln Val  
275 280 285

Val Gly Ile Ser Trp Tyr Leu Trp Ile Phe Val Val Ile Phe Leu Leu  
290 295 300

Leu Asn Val Asn Gly Trp His Thr Tyr Phe Trp Ile Ala Phe Ile Pro  
305 310 315 320

Phe Ala Leu Leu Leu Ala Val Gly Thr Lys Leu Glu His Val Ile Ala  
325 330 335

Gln Leu Ala His Glu Val Ala Glu Lys His Val Ala Ile Glu Gly Asp  
340 345 350

Leu Val Val Lys Pro Ser Asp Glu His Phe Trp Phe Ser Lys Pro Gln  
355 360 365

- 32 -

Ile Val Leu Tyr Leu Ile His Phe Ile Leu Phe Gln Asn Ala Phe Glu  
370 375 380

Ile Ala Phe Phe Phe Trp Ile Trp Val Thr Tyr Gly Phe Asp Ser Cys  
385 390 395 400

Ile Met Gly Gln Val Arg Tyr Ile Val Pro Arg Leu Val Ile Gly Val  
405 410 415

Phe Ile Gln Val Leu Cys Ser Tyr Ser Thr Leu Pro Leu Tyr Ala Ile  
420 425 430

Val Ser Gln Met Gly Ser Ser Phe Lys Lys Ala Ile Phe Glu Glu Asn  
435 440 445

Val Gln Val Gly Leu Val Gly Trp Ala Gln Lys Val Lys Gln Lys Arg  
450 455 460

Asp Leu Lys Ala Ala Ala Ser Asn Gly Asn Glu Gly Ser Ser Gln Ala  
465 470 475 480

Gly Pro Gly Pro Asp Ser Gly Ser Ala Pro Ala Ala Gly Pro  
485 490 495

Gly Ala Gly Phe Ala Gly Ile Gln Leu Ser Arg Val Thr Arg Asn Asn  
500 505 510

Ala Gly Asp Thr Asn Asn Glu Ile Thr Pro Asp His Asn Asn  
515 520 525

<210> 15

<211> 1782

<212> DNA

<213> Arabidopsis thaliana

<220>

<221> CDS

<222> (1)..(1779)

<220>

<221> misc\_feature

<222> (274)..(313)

<223> location of the amino acid sequence set forth in  
SEQ ID No:1

<220>

<221> misc\_feature

<222> (1327)..(1370)

<223> location of the amino acid sequence set forth in  
SEQ ID No: 2

<400> 15

atg gga atc atc gac ggt tct ttg ctt cgg cgg ttg att tgt ctc tgt 48  
Met Gly Ile Ile Asp Gly Ser Leu Leu Arg Arg Leu Ile Cys Leu Cys

1	5	10	15	
ctc tgg tgt ctt ctc ggt gga gga gtg acg gtg gtt acg gcg gag gat Leu Trp Cys Leu Leu Gly Gly Val Thr Val Val Thr Ala Glu Asp				96
20	25		30	
gag aag aaa gtg gta cat aaa cag ctt aat caa act ccg act tgg gct Glu Lys Lys Val Val His Lys Gln Leu Asn Gln Thr Pro Thr Trp Ala				144
35	40		45	
gtt gct gct gtt tgt act ttc ttc atc gtt tct gtt ctt ctt gaa Val Ala Ala Val Cys Thr Phe Phe Ile Val Val Ser Val Leu Leu Glu				192
50	55	60		
aaa ctt ctt cac aaa gtt gga aag gtt cta tgg gat ccg cac aag aca Lys Leu Leu His Lys Val Gly Lys Val Leu Trp Asp Arg His Lys Thr				240
65	70	75	80	
gct ctt ctt gac gct ttg gag aag atc aaa gca gag ctg atg gtt ctt Ala Leu Leu Asp Ala Leu Glu Lys Ile Lys Ala Glu Leu Met Val Leu				288
85	90		95	
gga ttc atc tct ttg ctt ctg aca ttt gga caa acc tac att ttg gat Gly Phe Ile Ser Leu Leu Leu Thr Phe Gly Gln Thr Tyr Ile Leu Asp				336
100	105		110	
att tgt atc cct tca cat gtt gct cgt acg atg ctc ccg tgt cct gct Ile Cys Ile Pro Ser His Val Ala Arg Thr Met Leu Pro Cys Pro Ala				384
115	120	125		
cct aac ttg aaa aag gag gat gat gac aat ggt gaa agt cac agg aga Pro Asn Leu Lys Lys Glu Asp Asp Asp Asn Gly Ser His Arg Arg				432
130	135	140		
ctc ttg tcg ttt gag cac aga ttt tta tct gga ggt gaa gca tct ccc Leu Leu Ser Phe Glu His Arg Phe Leu Ser Gly Gly Glu Ala Ser Pro				480
145	150	155	160	
act aaa tgc acg aag gag ggt tat gta gag ctt atc tct gcc gag gca Thr Lys Cys Thr Lys Glu Gly Tyr Val Glu Leu Ile Ser Ala Glu Ala				528
165	170		175	
ctc cat cag ttg cac atc ctt ata ttc ttc tta gcc att ttc cac gtt Leu His Gln Leu His Ile Leu Ile Phe Phe Leu Ala Ile Phe His Val				576
180	185		190	
ctt tac agc ttc tta act atg atg ctt gga agg ttg aag att cgc gga Leu Tyr Ser Phe Leu Thr Met Met Leu Gly Arg Leu Lys Ile Arg Gly				624
195	200	205		
tgg aag cat tgg gag aat gag aca tca tcc cat aat tac gag ttt tca Trp Lys His Trp Glu Asn Glu Thr Ser Ser His Asn Tyr Glu Phe Ser				672
210	215	220		
aca gac act tcc aga ttc agg cta act cat gaa aca tct ttt gtg aga				720

Thr Asp Thr Ser Arg Phe Arg Leu Thr His Glu Thr Ser Phe Val Arg				
225	230	235	240	
gct cac acc agt ttc tgg acc cgg att cca ttc ttt ttc tat gtt gga				768
Ala His Thr Ser Phe Trp Thr Arg Ile Pro Phe Phe Tyr Val Gly				
245		250	255	
tgc ttt ttc aga cag ttt ttc aga tcc gtt ggg aga act gac tat ttg				816
Cys Phe Phe Arg Gln Phe Phe Arg Ser Val Gly Arg Thr Asp Tyr Leu				
260	265		270	
aca ttg aga aat ggt ttc atc gct gtt cat tta gct cca gga agt caa				864
Thr Leu Arg Asn Gly Phe Ile Ala Val His Leu Ala Pro Gly Ser Gln				
275	280		285	
ttt aac ttc caa aaa tac att aaa aga tcg ttg gag gat gat ttc aag				912
Phe Asn Phe Gln Lys Tyr Ile Lys Arg Ser Leu Glu Asp Asp Phe Lys				
290	295		300	
gta gtc gtt gga gtc agc cct gtc ttg tgg gga tct ttt gtg cta ttc				960
Val Val Val Gly Val Ser Pro Val Leu Trp Gly Ser Phe Val Leu Phe				
305	310	315	320	
ctc ctc cta aat att gac ggt gag tat atg atg ttc atc ggc act gca				1008
Leu Leu Leu Asn Ile Asp Gly Glu Tyr Met Met Phe Ile Gly Thr Ala				
325		330	335	
ata ccg gtt att atc att tta gct gta ggg aca aag ctt caa gcg att				1056
Ile Pro Val Ile Ile Ile Leu Ala Val Gly Thr Lys Leu Gln Ala Ile				
340	345		350	
atg aca agg atg gct ctt ggt atc aca gat aga cat gcg gta gtt caa				1104
Met Thr Arg Met Ala Leu Gly Ile Thr Asp Arg His Ala Val Val Gln				
355	360		365	
gga atg ccg ctt gta caa ggc aac gat gag tat ttc tgg ttc ggt cgt				1152
Gly Met Pro Leu Val Gln Gly Asn Asp Glu Tyr Phe Trp Phe Gly Arg				
370	375		380	
ccc cat ttg att ctc cat ctc atg cat ttc gcc ttg ttt cag aac gca				1200
Pro His Leu Ile Leu His Leu Met His Phe Ala Leu Phe Gln Asn Ala				
385	390	395	400	
ttt cag atc act tat ttc ttc tgg ata tgg tat tcc ttt gga tca gat				1248
Phe Gln Ile Thr Tyr Phe Phe Trp Ile Trp Tyr Ser Phe Gly Ser Asp				
405	410		415	
tct tgc tac cat cct aat ttc aag att gca ctt gta aaa gta gcg att				1296
Ser Cys Tyr His Pro Asn Phe Lys Ile Ala Leu Val Lys Val Ala Ile				
420	425		430	
gct tta gga gta ttg tgt ctt tgc agc tac atc aca ctt cct ctt tac				1344
Ala Leu Gly Val Leu Cys Leu Cys Ser Tyr Ile Thr Leu Pro Leu Tyr				
435	440		445	

- 35 -

gca ctc gta act cag atg ggt tct cgg atg aaa aaa tcg gta ttc gat Ala Leu Val Thr Gln Met Gly Ser Arg Met Lys Lys Ser Val Phe Asp 450 455 460	1392
gaa caa acg tca aaa gca ctc aag aaa tgg aga atg gca gtg aag aag Glu Gln Thr Ser Lys Ala Leu Lys Lys Trp Arg Met Ala Val Lys Lys 465 470 475 480	1440
aag aaa ggt gtg aaa gcc act act aag aga cta ggt gga gat gga agt Lys Lys Gly Val Lys Ala Thr Thr Lys Arg Leu Gly Gly Asp Gly Ser 485 490 495	1488
gcg agc cct acg gca tcg aca gtt agg tct act tcg tct gta cgt tca Ala Ser Pro Thr Ala Ser Thr Val Arg Ser Thr Ser Ser Val Arg Ser 500 505 510	1536
ttg cag cgt tac aaa acc aca cca cat tcg atg aga tac gaa gga ctt Leu Gln Arg Tyr Lys Thr Thr Pro His Ser Met Arg Tyr Glu Gly Leu 515 520 525	1584
gac cct gaa aca tcg gat ctc gac aca gat aat gaa gct ttg act cct Asp Pro Glu Thr Ser Asp Leu Asp Thr Asp Asn Glu Ala Leu Thr Pro 530 535 540	1632
ccc aaa tct cct cca agc ttc gag ctt gtt gtg aaa gtt gaa cca aat Pro Lys Ser Pro Pro Ser Phe Glu Leu Val Val Lys Val Glu Pro Asn 545 550 555 560	1680
aag acc aat acc ggt gag act agc cgt gac act gaa act gat tct aaa Lys Thr Asn Thr Gly Glu Thr Ser Arg Asp Thr Glu Thr Asp Ser Lys 565 570 575	1728
gag ttc tct ttc gtc aag cct gct ccg agt aat gaa tca tctcaa gac Glu Phe Ser Phe Val Lys Pro Ala Pro Ser Asn Glu Ser Ser Gln Asp 580 585 590	1776
cggtga Arg	1782

<210> 16  
<211> 593  
<212> PRT  
<213> *Arabidopsis thaliana*

<400> 16  
Met Gly Ile Ile Asp Gly Ser Leu Leu Arg Arg Leu Ile Cys Leu Cys  
1 5 10 15  
Leu Trp Cys Leu Leu Gly Gly Val Thr Val Val Thr Ala Glu Asp  
20 25 30  
Glu Lys Lys Val Val His Lys Gln Leu Asn Gln Thr Pro Thr Trp Ala  
35 40 45

Val Ala Ala Val Cys Thr Phe Phe Ile Val Val Ser Val Leu Leu Glu  
50 55 60

Lys Leu Leu His Lys Val Gly Lys Val Leu Trp Asp Arg His Lys Thr  
65 70 75 80

Ala Leu Leu Asp Ala Leu Glu Lys Ile Lys Ala Glu Leu Met Val Leu  
85 90 95

Gly Phe Ile Ser Leu Leu Thr Phe Gly Gln Thr Tyr Ile Leu Asp  
100 105 110

Ile Cys Ile Pro Ser His Val Ala Arg Thr Met Leu Pro Cys Pro Ala  
115 120 125

Pro Asn Leu Lys Lys Glu Asp Asp Asn Gly Glu Ser His Arg Arg  
130 135 140

Leu Leu Ser Phe Glu His Arg Phe Leu Ser Gly Gly Glu Ala Ser Pro  
145 150 155 160

Thr Lys Cys Thr Lys Glu Gly Tyr Val Glu Leu Ile Ser Ala Glu Ala  
165 170 175

Leu His Gln Leu His Ile Leu Ile Phe Phe Leu Ala Ile Phe His Val  
180 185 190

Leu Tyr Ser Phe Leu Thr Met Met Leu Gly Arg Leu Lys Ile Arg Gly  
195 200 205

Trp Lys His Trp Glu Asn Glu Thr Ser Ser His Asn Tyr Glu Phe Ser  
210 215 220

Thr Asp Thr Ser Arg Phe Arg Leu Thr His Glu Thr Ser Phe Val Arg  
225 230 235 240

Ala His Thr Ser Phe Trp Thr Arg Ile Pro Phe Phe Tyr Val Gly  
245 250 255

Cys Phe Phe Arg Gln Phe Phe Arg Ser Val Gly Arg Thr Asp Tyr Leu  
260 265 270

Thr Leu Arg Asn Gly Phe Ile Ala Val His Leu Ala Pro Gly Ser Gln  
275 280 285

Phe Asn Phe Gln Lys Tyr Ile Lys Arg Ser Leu Glu Asp Asp Phe Lys  
290 295 300

Val Val Val Gly Val Ser Pro Val Leu Trp Gly Ser Phe Val Leu Phe  
305 310 315 320

Leu Leu Leu Asn Ile Asp Gly Glu Tyr Met Met Phe Ile Gly Thr Ala  
325 330 335

Ile Pro Val Ile Ile Leu Ala Val Gly Thr Lys Leu Gln Ala Ile

- 37 -

340

345

350

Met Thr Arg Met Ala Leu Gly Ile Thr Asp Arg His Ala Val Val Gln  
 355 360 365

Gly Met Pro Leu Val Gln Gly Asn Asp Glu Tyr Phe Trp Phe Gly Arg  
 370 375 380

Pro His Leu Ile Leu His Leu Met His Phe Ala Leu Phe Gln Asn Ala  
 385 390 395 400

Phe Gln Ile Thr Tyr Phe Phe Trp Ile Trp Tyr Ser Phe Gly Ser Asp  
 405 410 415

Ser Cys Tyr His Pro Asn Phe Lys Ile Ala Leu Val Lys Val Ala Ile  
 420 425 430

Ala Leu Gly Val Leu Cys Leu Cys Ser Tyr Ile Thr Leu Pro Leu Tyr  
 435 440 445

Ala Leu Val Thr Gln Met Gly Ser Arg Met Lys Lys Ser Val Phe Asp  
 450 455 460

Glu Gln Thr Ser Lys Ala Leu Lys Lys Trp Arg Met Ala Val Lys Lys  
 465 470 475 480

Lys Lys Gly Val Lys Ala Thr Thr Lys Arg Leu Gly Gly Asp Gly Ser  
 485 490 495

Ala Ser Pro Thr Ala Ser Thr Val Arg Ser Thr Ser Ser Val Arg Ser  
 500 505 510

Leu Gln Arg Tyr Lys Thr Thr Pro His Ser Met Arg Tyr Glu Gly Leu  
 515 520 525

Asp Pro Glu Thr Ser Asp Leu Asp Thr Asp Asn Glu Ala Leu Thr Pro  
 530 535 540

Pro Lys Ser Pro Pro Ser Phe Glu Leu Val Val Lys Val Glu Pro Asn  
 545 550 555 560

Lys Thr Asn Thr Gly Glu Thr Ser Arg Asp Thr Glu Thr Asp Ser Lys  
 565 570 575

Glu Phe Ser Phe Val Lys Pro Ala Pro Ser Asn Glu Ser Ser Gln Asp  
 580 585 590

Arg

<210> 17

<211> 1629

<212> DNA

<213> Arabidopsis thaliana



- 39 -

Gly Leu Glu Gln Leu Leu Arg Phe Leu Phe Val Leu Gly Ile Thr His 145                    150                    155                    160	
gtt cta tac agt ggc att gcc att ggt tta gcc atg agc aag att tac Val Leu Tyr Ser Gly Ile Ala Ile Gly Leu Ala Met Ser Lys Ile Tyr 165                    170                    175	528
agt tgg aga aaa tgg gaa gcc caa gcg atc ata atg gct gaa tca gat Ser Trp Arg Lys Trp Glu Ala Gln Ala Ile Ile Met Ala Glu Ser Asp 180                    185                    190	576
atc cac ctt tgt ttc ctg cgga ttt aga ggc tcc ata cga aag tct Ile His Leu Cys Phe Leu Arg Gln Phe Arg Gly Ser Ile Arg Lys Ser 195                    200                    205	624
gac tac ttc gca ctt cgga tta ggt ttc ctc act aaa cat aat ttg cca Asp Tyr Phe Ala Leu Arg Leu Gly Phe Leu Thr Lys His Asn Leu Pro 210                    215                    220	672
ttt aca tac aac ttc cat atg tat atg gta cgga acg atg gaa gat gag Phe Thr Tyr Asn Phe His Met Tyr Met Val Arg Thr Met Glu Asp Glu 225                    230                    235                    240	720
ttt cat ggc att gtt gga att agc tgg cca ctt tgg gtt tac gct ata Phe His Gly Ile Val Gly Ile Ser Trp Pro Leu Trp Val Tyr Ala Ile 245                    250                    255	768
gta tgc atc tgc ata aat gtt cat ggc ctg aat atg tac ttt tgg ata Val Cys Ile Cys Ile Asn Val His Gly Leu Asn Met Tyr Phe Trp Ile 260                    265                    270	816
tca ttc gtt cct gcc att ctt gtc atg ttg gta acc aaa ctt gag Ser Phe Val Pro Ala Ile Leu Val Met Leu Val Gly Thr Lys Leu Glu 275                    280                    285	864
cat gtt gtc tcc aag ctt gct ctc gag gtt aag gag cag cag aca ggc His Val Val Ser Lys Leu Ala Leu Glu Val Lys Glu Gln Gln Thr Gly 290                    295                    300	912
aca tct aat ggg gct caa gtc aaa cca cgt gat ggg ctc ttc tgg ttt Thr Ser Asn Gly Ala Gln Val Lys Pro Arg Asp Gly Leu Phe Trp Phe 305                    310                    315                    320	960
ggg aaa cca gaa att ctg cta cgga ttg ata caa ttt atc att ttt cag Gly Lys Pro Glu Ile Leu Leu Arg Leu Ile Gln Phe Ile Ile Phe Gln 325                    330                    335	1008
aat gca ttt gaa atg gca aca ttc atc tgg ttc ttg tgg gga atc aag Asn Ala Phe Glu Met Ala Thr Phe Ile Trp Phe Leu Trp Gly Ile Lys 340                    345                    350	1056
gaa aga tct tgc ttc atg aag aac cat gtg atg ata tca agc cgga cta Glu Arg Ser Cys Phe Met Lys Asn His Val Met Ile Ser Ser Arg Leu 355                    360                    365	1104

- 40 -

att tct ggg gtt ctc gtt cag ttc tgg tgt agt tat ggc act gtg cct Ile Ser Gly Val Leu Val Gln Phe Trp Cys Ser Tyr Gly Thr Val Pro 370 375 380	1152
ctc aat gta atc gtt act cag atg gga tct cggt cat aag aaa gct gtg Leu Asn Val Ile Val Thr Gln Met Gly Ser Arg His Lys Lys Ala Val 385 390 395 400	1200
ata gca gag agc gta aga gac tca ctt cac agt tgg tgc aag aga gtg Ile Ala Glu Ser Val Arg Asp Ser Leu His Ser Trp Cys Lys Arg Val 405 410 415	1248
aaa gag agg tct aag cac acg aga tca gtg tgt tcc ctt gac aca gca Lys Glu Arg Ser Lys His Thr Arg Ser Val Cys Ser Leu Asp Thr Ala 420 425 430	1296
aca ata gac gag aga gac gag atg aca gtg ggg aca ttg tct agg agc Thr Ile Asp Glu Arg Asp Glu Met Thr Val Gly Thr Leu Ser Arg Ser 435 440 445	1344
tca tcg atg act tca ctg aat cag att acc ata aac tcc ata gac caa Ser Ser Met Thr Ser Leu Asn Gln Ile Thr Ile Asn Ser Ile Asp Gln 450 455 460	1392
gca gag tct ata ttc gga gca gca gct tca tcc agc agt cct caa gat Ala Glu Ser Ile Phe Gly Ala Ala Ser Ser Ser Pro Gln Asp 465 470 475 480	1440
gga tac acg tcg agg gtg gaa gaa tat ctg tct gaa aca tac aat aac Gly Tyr Thr Ser Arg Val Glu Glu Tyr Leu Ser Glu Thr Tyr Asn Asn 485 490 495	1488
atc ggt tcg ata ccg cct tta aac gat gag att gag att gag att gaa Ile Gly Ser Ile Pro Pro Leu Asn Asp Glu Ile Glu Ile Glu Ile Glu 500 505 510	1536
ggt gaa gaa gat aat gga ggg aga gga agt ggg agt gat gag aat aac Gly Glu Glu Asp Asn Gly Gly Arg Gly Ser Gly Ser Asp Glu Asn Asn 515 520 525	1584
ggt gat gct gga gaa aca ctt ctt gag ttg ttt agg agg act tga Gly Asp Ala Gly Glu Thr Leu Leu Glu Leu Phe Arg Arg Thr 530 535 540	1629
<210> 18	
<211> 542	
<212> PRT	
<213> Arabidopsis thaliana	
<400> 18	
Met Glu His Met Met Lys Glu Gly Arg Ser Leu Ala Glu Thr Pro Thr 1 5 10 15	
Tyr Ser Val Ala Ser Val Val Thr Val Leu Val Phe Val Cys Phe Leu	

- 41 -

20	25	30
Val Glu Arg Ala Ile Tyr Arg Phe Gly Lys Trp Leu Lys Lys Thr Arg		
35	40	45
Arg Lys Ala Leu Phe Thr Ser Leu Glu Lys Met Lys Glu Glu Leu Met		
50	55	60
Leu Leu Gly Leu Ile Ser Leu Leu Ser Gln Ser Ala Arg Trp Ile		
65	70	75
Ser Glu Ile Cys Val Asn Ser Ser Leu Phe Asn Ser Lys Phe Tyr Ile		
85	90	95
Cys Ser Glu Glu Asp Tyr Gly Ile His Lys Lys Val Leu Leu Glu His		
100	105	110
Thr Ser Ser Thr Asn Gln Ser Ser Leu Pro His His Gly Ile His Glu		
115	120	125
Ala Ser His Gln Cys Gly His Gly Arg Glu Pro Phe Val Ser Tyr Glu		
130	135	140
Gly Leu Glu Gln Leu Leu Arg Phe Leu Phe Val Leu Gly Ile Thr His		
145	150	155
160		
Val Leu Tyr Ser Gly Ile Ala Ile Gly Leu Ala Met Ser Lys Ile Tyr		
165	170	175
Ser Trp Arg Lys Trp Glu Ala Gln Ala Ile Ile Met Ala Glu Ser Asp		
180	185	190
Ile His Leu Cys Phe Leu Arg Gln Phe Arg Gly Ser Ile Arg Lys Ser		
195	200	205
Asp Tyr Phe Ala Leu Arg Leu Gly Phe Leu Thr Lys His Asn Leu Pro		
210	215	220
Phe Thr Tyr Asn Phe His Met Tyr Met Val Arg Thr Met Glu Asp Glu		
225	230	235
240		
Phe His Gly Ile Val Gly Ile Ser Trp Pro Leu Trp Val Tyr Ala Ile		
245	250	255
Val Cys Ile Cys Ile Asn Val His Gly Leu Asn Met Tyr Phe Trp Ile		
260	265	270
Ser Phe Val Pro Ala Ile Leu Val Met Leu Val Gly Thr Lys Leu Glu		
275	280	285
His Val Val Ser Lys Leu Ala Leu Glu Val Lys Glu Gln Gln Thr Gly		
290	295	300
Thr Ser Asn Gly Ala Gln Val Lys Pro Arg Asp Gly Leu Phe Trp Phe		
305	310	315
320		

- 42 -

Gly Lys Pro Glu Ile Leu Leu Arg Leu Ile Gln Phe Ile Ile Phe Gln  
 325 330 335  
 Asn Ala Phe Glu Met Ala Thr Phe Ile Trp Phe Leu Trp Gly Ile Lys  
 340 345 350  
 Glu Arg Ser Cys Phe Met Lys Asn His Val Met Ile Ser Ser Arg Leu  
 355 360 365  
 Ile Ser Gly Val Leu Val Gln Phe Trp Cys Ser Tyr Gly Thr Val Pro  
 370 375 380  
 Leu Asn Val Ile Val Thr Gln Met Gly Ser Arg His Lys Lys Ala Val  
 385 390 395 400  
 Ile Ala Glu Ser Val Arg Asp Ser Leu His Ser Trp Cys Lys Arg Val  
 405 410 415  
 Lys Glu Arg Ser Lys His Thr Arg Ser Val Cys Ser Leu Asp Thr Ala  
 420 425 430  
 Thr Ile Asp Glu Arg Asp Glu Met Thr Val Gly Thr Leu Ser Arg Ser  
 435 440 445  
 Ser Ser Met Thr Ser Leu Asn Gln Ile Thr Ile Asn Ser Ile Asp Gln  
 450 455 460  
 Ala Glu Ser Ile Phe Gly Ala Ala Ala Ser Ser Ser Ser Pro Gln Asp  
 465 470 475 480  
 Gly Tyr Thr Ser Arg Val Glu Glu Tyr Leu Ser Glu Thr Tyr Asn Asn  
 485 490 495  
 Ile Gly Ser Ile Pro Pro Leu Asn Asp Glu Ile Glu Ile Glu Ile Glu  
 500 505 510  
 Gly Glu Glu Asp Asn Gly Gly Arg Gly Ser Gly Ser Asp Glu Asn Asn  
 515 520 525  
 Gly Asp Ala Gly Glu Thr Leu Leu Glu Leu Phe Arg Arg Thr  
 530 535 540  
  
 <210> 19  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence:  
 Oligonucleotide  
  
 <400> 19  
 atgtcggaca aaaaagggtt

<210> 20  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 20  
atgctaccac acgcagatcg

20

<210> 21  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 21  
acagagacca cctccttgaa a

21

<210> 22  
<211> 22  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 22  
cagaaaacttg tctcatccct gg

22

<210> 23  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 23  
aagaactgcc tgaagaaggc

20

<210> 24

<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 24  
caccacccttc atgatgctca

20

<210> 25  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 25  
ttccagcacc ggcacaagaa

20

<210> 26  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 26  
tggacctctt catgttcgat cccatctg

28

<210> 27  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 27  
cctgacgctg ttccagaatg cgtttca

27

<210> 28  
<211> 20  
<212> DNA  
<213> Artificial Sequence

- 45 -

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 28  
acttctgcag gtcgactcta

20

<210> 29  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 29  
aagatcaaga tgaggacgtg gaagtcgtgg

30

<210> 30  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 30  
aggctgaacc actggggcgc ctctcaccac

30

<210> 31  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 31  
caagtatatg atgcgcgctc tagaggatga

30

<210> 32  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:

**Oligonucleotide**

<400> 32  
aggtttacc actaagtctc cttcaatggc 30

<210> 33  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 33  
gatcattcaa gacttaggct cactcatgag 30

<210> 34  
<211> 30  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 34  
aacagcaagg aagattacaa atgatgccca 30

<210> 35  
<211> 18  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 35  
ggattaagat ctaatggc 18

<210> 36  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 36

caaagatctt catttcttaa aag

23

<210> 37  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 37  
gcggatccat gtcggacaaa aaagg

25

<210> 38  
<211> 25  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 38  
gcggatccctc atccctggct gaagg

25

<210> 39  
<211> 23  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 39  
ggatccacca tggccacaag atg

23

<210> 40  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 40  
ggatccttag tcaatatcat tagc

24

<210> 41  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 41  
gcggatccat gggcacgga ggagaag

27

<210> 42  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence:  
Oligonucleotide

<400> 42  
gcggatcctc agttgttatg atcagga

27